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(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES

(57) Abstract

The invention provides compositions comprising uscharin and the use of uscharin to combat cell proliferation for example in the treatment of cancer. Administration of uscharin may kill or reduce the growth rate of cancer cells and may also be of application in other medical conditions presenting symptoms of excessive or uncontrolled cell proliferation. The composition may be administered by any convenient route and formulated accordingly. The composition may be administered locally or generally and may be suitably dissolved and/or suspended in a pharmaceutically acceptable liquid carrier medium.

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PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES

This invention relates to a composition comprising the cardenolide glycoside uscharin.

Plants of the family *Asclepidaceae* are known to be extremely poisonous. Such plants have a history of use in folk medicines in those areas where they occur naturally, for example in South East Asia and Africa. Two of the best known representatives of the Asclepiadaceae are *Calotropis gigantea* and *Calotropis procera*. Extracts from *Calotropis procera* plants have traditionally been used as an abortifacient, for infanticide, for rheumatic pain and to produce a purgative.

The stems, flowers and leaves of plants from the family *Asclepiadaceae* (including *Calotropis gigantea* and *Calotropis procera)* are known to contain certain compounds known as cardenolides. In several species substantial amounts of cardenolides have been found to be concentrated in the latex (Roeske et al, in Biochemical Interactions Between Plants and Insects published in Volume 10 of Recent Advances in

Phytochemistry, Plenum Press, New York (ed. Wallace), Seiber et al, Phytochemistry 21:2343 (1982), Seiber et al, in Isopentoids in Plants, Academic Press (ed Nes, 1984) and Seiber et al, in J. Chem. Ecol. 6:321 (1980)). The natural production of cardenolides in Ascelopias curassavia has been reported by Groeneveld et al in Phytochemistry 29(11):3479-3486 (1990). Examples of cardenolide glycosides found in *C. procera* are voruscharin, uscharin, uscharidin, calotropin, calactin, calotoxin, and calotropagenin. Formula I shows the chemical structure of these cardenolides.

It has now been found that the cardenolide uscharin is particularly useful for medical purposes. Whilst uscharin has been isolated and its chemical structure determined, no utility for this compound has previously been reported.

The present invention thus provides a composition comprising uscharin, the analogues and salts thereof as active ingredient together with a pharmaceutically acceptable carrier or excipient.

Further, the present invention also provides the use of uscharin, the analogues and salts thereof for medical (including veterinary) purposes.

Previously, certain cardenolide glycosides such as calotropin and uzarigenin have been noted to have cytotoxic activity against primate tumour cells. Certain cardenolide glycosides from the *Asclepiadaceae* family share structural and pharmacological similarities with the *Digitalis* cardiac glycosides. Whilst we do not wish to be bound by theoretical considerations it is believed that the cytotoxicity of some cardenolide glycosides is related to the inhibition of the plasma membrane bound Na^+/K^+ ATPase (ie analogous to the manner in which *Digitalis* cardiac glycosides exert their toxic effects). However, it has also been shown that whilst some cardenolide glycosides are cytotoxic to cell cultures they have no <u>in vivo</u> tumour-inhibiting activity. This is true of calotropin and uzarigenin.

It has never previously been proposed that uscharin would be useful for medical applications. The inventors' results have shown that at 1mg/ml a primary

extract of *Calotropis gigantea* known as CGE-1 does have tumour inhibiting activity in rats (weighing about 200g) and does not lead to the death of the test animals.

Typically, the use of uscharin according to the present invention is to combat cell proliferation for example in the treatment of cancer. Thus administration of uscharin may kill or reduce the growth rate of cancer cells and may also be of application in other medical conditions presenting symptoms of excessive or uncontrolled cell proliferation.

The word "combat" is used herein to refer to treatment of an existing condition so as to alleviate or reverse the symptoms of the condition in an affected human or animal and to prevent such a condition in a healthy human or animal.

The composition according to the present invention may be administered by any convenient route and mention may be made of enteral, parenteral, topical administration and the composition will be formulated accordingly. Conveniently, the composition may be administered locally to the affected site, generally by means of injection. Thus the uscharin will be suitably dissolved and/or suspended in a pharmaceutically acceptable liquid carrier medium, which will generally be aqueous-based, for example an isotonic solution. Alternatively, the composition according to the invention may be taken orally.

Formulations for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers,

bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemoraneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

The dose will depend on a number of factors known to the skilled physician including the severity of the conditions, the identity of the recipient; and also the efficacy and toxicity of the particular composition which is being administered. Generally doses in the range 0.1-100 mg/kg body weight may be used, particularly 1-10 mg/kg. The frequency of administration will vary depending on the rate of metabolism or excretion of the administered compound, but may be repeated daily, optionally as two or more sub-doses. Unit doses of 20 to 500 mg, preferably 100 to 400 mg may be used.

A single dosage may be given daily or smaller quantities or dosage units may be given at intervals throughout a 24 hour period, for example dosage units given 2, 3 or 4 times throughout the day.

Any type of cancer or condition involving cell proliferation may be treated by the present invention. Uscharin is especially useful for the treatment of

cancers such as leukaemia, non-small cell lung cancer, small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostrate cancer, and breast cancer. However the invention is not limited to treatment of these specific conditions since uscharin is believed to be of general effect.

Cancers where uscharin is particularly efficacious include ovarian cancer and skin cancer.

Uscharin may by produced by any convenient method, for example by chemical synthesis. Alternatively the uscharin may be conveniently extracted and purified from organisms (for example plants of the family *Asclepiadacaeae*) which produce uscharin naturally. It is also envisaged that uscharin may be manufactured using genetically engineered micro-organisms, plants or animals or may be made using cell-culture or other biotechnological techniques.

Further, the present invention also provides the use of a composition as described above for medical purposes, for example to combat conditions in which cell proliferation is undesirable (eg cancer).

In another aspect, the present invention provides the use of uscharin in the manufacture of a medicament. Generally such medicament would be of use to combat cancer and other conditions where cell proliferation is undesirable.

In a further aspect, the present invention provides a method of treatment of a human or non-human animal body, said method comprising administering to said body a composition as described above.

The present invention is now further described by means of the following, non-limiting Examples.

EXAMPLE 1

PREPARATION OF USCHARIN EXTRACT

(i) ISOLATION OF CGE-1

Leaves of *Calotropis gigantea* (500g) were Soxhlet extracted initially with petroleum ether (60-80), then ethyl acetate and finally methanol. The cell culture bioassays showed that the ethyl acetate fraction contained cytotoxic activity. The ethyl acetate extract was subjected to vacuum liquid chromatography (VLC) on silica gel 60H (Merck). Elution was initiated with petroleum ether (60-80) and proceeded with petroleum ether containing progressively greater amounts of ethyl acetate through to ethyl acetate only. Elution was then continued with ethyl acetate containing progressively greater amounts of methanol.

Samples of the fraction were collected and prepared for cytotoxicity testing by solubilisation in 0.1% Tween.

The greatest cytotoxic activity (ED_{50} <0.10µg/ml) was found in the 70-80% ethyl acetate in petroleum ether fractions. The cytotoxic compound CGE-1 (72.0 mg) (ED_{50}< 0.09µg/ml) was isolated as a white semi-crystalline precipitate from this fraction.

(ii) ISOLATION OF CGE-2

Another less cytotoxic compound, CGE-2 (101.0mg) (ED_{50} <8.0µg/ml) was isolated from the 100% ethyl acetate

fraction as a semi-crystalline precipitate.

(iii) PROPERTIES OF CGE-1

White powder, found 587.2511, $C_{31}H_{41}NO_8S$ requires 587,2553. $[\alpha]_0$ + 10.0° (c.0.1,CH_3OH_4) IR V_{max} CM^{-1}: 3465, 2960, 2920, 2840, 2720, 1735, 1730, 1705, 1625, 1540, 1160, 1110, 1060, 1040. EIMS m/z (rel. int.) 587 [M+] (4.0), 233 (14.9), 215 (8.6), 187 (9.8), 183

ACTIVITY OF CGE-1

At a concentration of 1 mg/ml, CGE-1 has a tumor inhibiting activity in rats weighing approximately 200g and does not lead to the death of the rat.

CGE-1 was found to contain Uscharin.

EXAMPLE 2

Isolation of Uscharin from *Calotropis Gigantea* leaves.

EXTRACTION

The plant material was minced to a fine powder in a bench grinder. The powder was extracted in a Soxhlet with petroleum ether (60-80) and the ethyl acetate, until exhaustion. The ethyl acetate fraction was concentrated to dryness using a rotary evaporator.

FRACTIONATION

Vacuum Liquid Chromatography was used for the initial fractionation of the crude extract Silica gel 60H

(Merck) was packed in a scintered funnel under vacuum to give a compact column. The crude extract, adsorbed in silica, was applied to the column. Elution was initiated with petroleum ether and proceeded with petroleum ether containing progressively greater amounts of ethyl acetate than with ethyl acetate through to methanol. The fractions were concentrated using a rotary evaporator. 10 mg of each fraction were prepared for cytotoxicity testing (see MTT assay for method) by solubilisation in DMSO. The fraction containing the greatest cytotoxic activity was subjected to a sephadex column to remove any remaining chlorophyll.

SEPHADEX COLUMN

The fraction was dissolved in a minimum volume of chloroform and applied to a column containing lipophilic sephadex LH-20 (Sigma) which had been packed in chloroform. Elution was with chloroform, chloroform with methanol and methanol. As before fraction were dried and tested for activity. The fraction with the greatest activity was further fractionated with a silica gel column.

SILICA GEL COLUMN

The fraction was dissolved in a minimum volume of chloroform and applied to a column containing silica gel (packed in chloroform). Elution was with chloroform, chloroform with methanol and methanol. This column yielded a fraction of almost pure uscharin. The pure compound was obtained from this fraction by preparative TLC.

PREPARATIVE TLC

The fraction was spotted onto glass silica gel plates. The plates were run in ethyl acetate and methanol (97:3). The silica was scratched from the plate and the uscharin eluted with ethyl acetate.

Once the compound had been isolated, its identity was confirmed by spectroscopic techniques.

EXAMPLE 3

CYTOTOXICITY BIOASSAY OF USCHARIN

Cytotoxicity bioassays were performed. The cell line used was a human ovarian small cell carcinoma SCC Wm 1(151) which was grown as a monolayer in Dulbecco's Modified Eagles Medium (Gibco) supplemented with 5% foetal calf serum (v/v), sodium pyruvate (1mM), penicillin (50IU/ml) and streptomycin (50µg/ml). Cultures were maintained in a humidified atmosphere of 5% CO_2/95% air at 37°.

Single cell suspensions were obtained by trypsinisation of the monolayer cultures and an equal number of cells (10^3-10^4 depending on the cell line) was inoculated into each 33mm² well of a 96 well plate in 190µl of culture medium. The plates were incubated for 24 hours to allow cells to adhere. At this point 10µl of an appropriate concentration of plant extract or control solvent was added to each well. The cells were exposed to the drug for 3 days after which the medium was removed, the monolayers washed with PBS and fresh medium added. This was repeated 24 hours later. Following a further 24 hours incubation 100µg (50µl of

2mg/ml in PBS) MTT (3-(4,5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was added to each well and the cells were incubated at 37°C for 4 hours. Plates were then processed using a modified version (Carmichael et al, 1987) of the assay first described by Mossman, T.(1983), where DMSO was used in preference to acid isopropanol to solubilise the formazan crystals. The contents of each well were mixed and the plate was read immediately at 540nm on a Flow Titertek Multiscan MCC/340 Mk 11 plate reader. Cells were set up in parallel at two densities, 10^3 and 2 x 10^3 cells/well, and the results from an assay were discarded if the ratio of the OD readings of the two densities was greater than 2.25:1 or less than 1.75:1.

The results obtained were as shown in Fig. 1

EXAMPLE 4

IN VITRO SCREENING OF USCHARIN

Uscharin was obtained as in Example 2 and was subjected to in vitro cell screening at the National Cancer Institute (NCI), USA in respect of a panel of cancel cell types organised into subpanels representing leukemia, lung cancers, colon cancer, cancer of the central nervous system, melanoma, ovarian cancer, renal cancer, and in some cases prostate cancer and breast cancer also.

The standard NCI methodology which was employed is described in Michael R Boyd, Principles and Practices of Oncology, Vol. 3, No. 10 (Oct. 1989) and Monks A. et al., Journal of the National Cancer Institute, Vol. 83, No. 11, (5th June, 1991).

The results of two separate screening experiements carried out using uscharin are given in Tables 1 and 2.

The data are derived from Dose-Response Curves and two typical curves for leukemia and colon cancer are given for illustrative purposes in Figures 1 and 2 attached hereto.

The Dose-Response Curve is created by plotting the Calculated Percent Growth (PG) of each cell line against the $\log_{(10)}$ of the corresponding drug concentration. The cell line curves are grouped by cell type, or subpanel. Mean $\text{Log}_{(10)}$ concentrations for all cell lines tested are calculated at three points: where the test compound achieved 50% inhibition of cell growth (GI_{50}), where the test compound achieved 0% cell growth or total growth inhibition (TGI), and where the test compound achiveved 50% cell kill or 50% lethal concentration (LC_{50}). Reference lines are shown at the percent growth values of +50 (GI_{50}), 0 (TGI) and -50 (LC_{50}).

Percentage Growth (PG) - of the compound on a cell line is currently calculated according to one of the following expressions:

If (Mean OD(test) - Mean OD(tzero) >= 0, then

PG = 100 x (Mean OD(test) - Mean OD(tzero)/(mean OD(ctrl) - Mean OD(tzero)

If (Mean OD(test - Mean OD(tzero) < 0, then PG = 100 x (Mean OD(test) - Mean OD(tzero)/Mean OD(tzero)

Where:

Mean OD (tzero) = The average of optical density measurements of SRB-derived colour just before exposure of cells to the test compound.

Mean OD (test) = The average of optical density measurements of SRB-derived colour after 48 hours with no exposure of cells to the test compound.

Mean OD (ctrl) = The average of optical density measurements of SRB-derived colour after 48 hours with no exposure of cells to the test compound.

It is clear from the results given in Tables 1 and 2 that uscharin has an inhibitory effect on the growth of a wide variety of cancer cell lines in vitro.

EXAMPLE 5

IN VITRO SCREENING OF USCHARIDIN

Uscharidin was also subjected to in vitro cell screening in the manner described in Example 4. Results are given in Table 3 and Figure 3, and these show that Uscharidin also exerts an inhibitory effect on a variety of cancer cell lines in vitro.

EXAMPLE 6

IN VITRO SCREENING OF CALOTOXIN

Calotoxin was also subjected to in vitro cell screening in the manner described in Example 4. Results are given in Table 4 and Figure 4, which show that calotoxin also exerts an inhibitory effect on a variety of cancer cell lines in vitro.

EXAMPLE 7

IN VITRO EXPERIEMENT WITH USCHARIN IN NUDE MICE

The SCCI cells (human tumour cell line) where grown (1 x 10^5/ml seeding density) in 25 ml RPMI 1640 (10% foetal calf serum, 5% glutamine) in 75 cm^2 tissue culture flasks. The cells were harvested at log growth phase (5 days approximately) and washed once in saline before injection into the mice.

The "nude" mice (BALB/c nude) are reared and contained within a sealed isolator. The mice were injected with 1 x 10^7 cells subcut on the back, right hand side near the shoulder blades. After 7 days the mice were split randomly into the study groups (10-15 animals per group). Each was then treated with a different regime, the variable being time between injections and dose of drug at each injection, control groups were also included in the overall plan of the experiement.

During the trial a daily check was made on the animals and any animal removed if the tumour size became too large (>5-7% total body weight) or if the animal is showing signs of distress. Additional to this the

tumour should be assessed every 3-4 days by an independent observer and the result recorded. Once an animal is removed from the study the tumour size, volume and weight was determined and the tumour stored for further cytological study. The reason for the animals removal from the study was also recorded, if this was not due to tumour size. The results are shown in the following tables.

Using nude mice injected with 10^7 SCC-1 cells injected on day 0 and drug treatment started on day 9.

GROUP NO. 1

0.1 mg CGE-1/ Animal/ 5 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	27	4356.4	1.7492	64.8	22.41	1
B	55	-	NONE	-	-	5
C	30	4141.3	2.5658	85.5	45.28	1
D	30	299.8	1.8196	60.7	52.24	1
E	37	2752.8	1.5783	42.7	33.37	1
F	55	-	NONE	-	-	5
G	55	-	NONE	-	-	5
H	55	-	NONE	-	-	5
I	33	3414.9	1.8805	57.0	28.69	1
J	55	-	NONE	-	-	5
K	37	828.9	0.6773	18.3	8.19	2
L	27	2223.8	1.6854	62.4	48.92	1
M	27	1556.2	0.7728	28.6	5.45	1
N	27	3457.9	1.9394	71.8	52.94	1
O	55	-	NONE	-	-	5
MEAN		2559.11	1.6298	54.64	33.05	
S.D.		1437.34	0.5844	21.20	18.29	

GROUP NO. 2

0.1 mg CGE-1/ Animal/ 10 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	27	2993.1	2.0570	76.2	49.92	1
B	55	-	NONE	-	-	5
C	55	-	NONE	-	-	5
D	55	-	NONE	-	-	5
E	55	664.8	0.4333	7.9	17.91	5
F	55	3148.8	2.0378	37.1	16.96	5
G	55	134.4	0.1285	2.3	8.17	5
H	55	-	NONE	-	-	5
I	55	-	NONE	-	-	5
J	55	-	NONE	-	-	5
K	55	-	NONE	-	-	5
L	55	-	NONE	-	-	5
M	26	2025.9	1.3238	50.9	6.90	3
N	30	1548.8	1.2677	42.3	10.79	1
O	30	544.1	0.3827	12.8	25.29	4
MEAN		1579.99	1.0901	32.79	19.42	
S.D.		1201.27	0.7933	26.68	14.9	

GROUP NO. 3

0.5 mg CGE-1/ Animal/ 5 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	55	-	NONE	-	-	5
B	55	219.6	0.2082	3.8	18.18	5
C	55	-	NONE	-	-	5
D	19	1494.7	1.1889	62.6	2.33	3
	19	203.2	0.0948	5.0	-	
E	19	-	NONE	-	-	3
F	23	3912.0	2.5341	110.2	13.13	1
G	28	4463.2	2.5717	91.8	23.42	1
H	37	-	NONE	-	-	2
I	28	1666.5	1.0930	39.0	12.96	1
J	19	23.7	0.0038	0.2	-	3
K	33	1457.9	1.2546	38.0	19.22	1
L	29	1532.5	0.8926	30.8	12.49	1
M	29	2972.3	1.6348	56.4	17.79	1
N	37	537.9	0.4997	13.5	9.70	2
O	37	-	NONE	-	-	2
MEAN		1848.36	1.1976	45.12	14.36	
S.D.		1504.32	0.8738	36.61	6.18	

GROUP NO. 4

0.5 mg CGE-1/ Animal/ 10 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	28	1482.1	1.1211	40.0	28.48	1
B	27	3499.1	2.5087	92.9	32.54	1
C	42	1930.3	1.4088	33.5	13.58	1
D	42	2177.3	1.5067	35.9	17.14	1
E	55	-	NONE	-	-	5
F	27	6882.3	3.1626	117.1	42.37	1
G	33	760.9	0.7467	22.6	50.31	1
H	55	-	NONE	-	-	5
I	55	-	NONE	-	-	5
J	55	-	NONE	-	-	5
K	55	64.5	0.1127	2.0	17.78	5
L	29	-	NONE	-	-	2
M	55	-	NONE	-	-	5
N	23	4929.6	2.6126	113.6	37.52	1
O	55	-	NONE	-	-	5
MEAN		2715.76	1.6475	57.2	29.97	
S.D.		2272.64	1.0344	44.08	13.18	

GROUP NO. 5

CONTROL (0.1 ml Saline/ Animal/ 5 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	55	-	NONE	-	-	5
B	55	-	NONE	-	-	5
C	55	-	NONE	-	-	5
D	55	-	NONE	-	-	5
E	23	4570.9	2.4227	105.3	35.2	1
F	50	3138.3	1.9475	39.0	4.43	1
G	55	-	NONE	-	-	5
H	55	-	NONE	-	-	5
I	3	-	NONE	-	-	3
J	23	5493.0	3.1602	137.4	59.07	1
K	28	2500.7	1.8958	67.7	6.68	1
L	28	3246.9	1.9716	70.4	31.86	1
M	55	-	NONE	-	-	5
N	28	4120.3	2.2965	82.0	46.07	1
O	55	-	NONE	-	-	5
MEAN		3845.02	2.2707	83.63	30.55	
S.D.		1093.88	0.4797	34.01	21.59	

1 NOTES:-
2 REASONS:
3
4 (1) Removed due to tumour size.
5 (2) Removed due to another illness.
6 (3) Found dead in cage.
7 (4) Removed because the tumour was about to rupture.
8 (5) Removed at end of the experiment.
9

TABLE 5

Table 5 gives a summary of the results.

	Tumour Growth (mg/day)	% Necrosis*	% Mortality at 40 days
Group 1 (0.1mg/5 days)	54.6 ± 21.1	33.1 ± 18.3	84
Group 2 (0.1mg/10 days)	32.8 ± 26.7	19.4 ± 14.9	55
Group 3 (0.5mg/5 days)	45.1 ± 36.6	14.4 ± 6.2	90
Group 4 (0.5mg/10 days)	57.2 ± 44.1	30.0 ± 13.2	62
Control	83.6 ± 34.0	30.6 ± 21.6	100

* from histological examination
Values are means ±SD, n=15

From these results it can be seen that a reduction in percentage mortallity due to the cancer cells of up to 45% can be achieved by administration of the compound of the invention (Uscharin).

CLAIMS

1. A composition comprising uscharin or analogues or salts thereof as active ingredient together with a pharmaceutically acceptable carrier or excipient.

2. The use of uscharin, analogues or salts thereof for medical (including veterinary) purposes.

3. The use of uscharin as claimed in the preparation of a medicament.

4. A composition as claimed in Claim 1 or 2 wherein the uscharin is suspended or dissolved in an acceptable liquid carrier medium.

5. A composition as claimed in Claim 4 wherein the carrier medium is aqueous based.

6. A use as claimed in Claims 2 or 3 wherein 0.1-100 uscharin per kg body weight is used.

7. A method of treatment of a human or non-human animal body, said method comprising administering to said body a composition comprising uscharin.

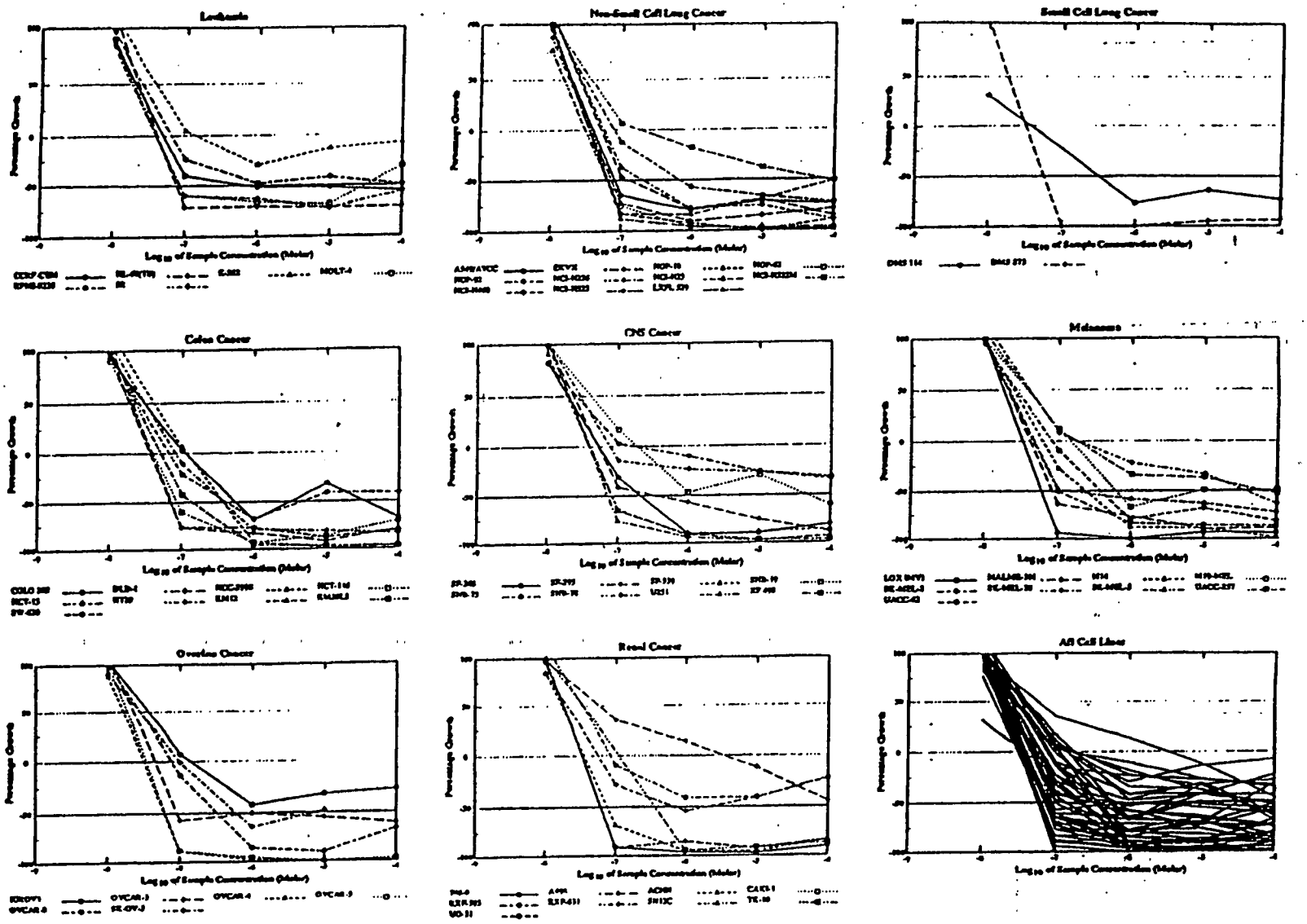
8. A method as claimed in Claim 7 wherein a unit dose of composition comprises between 20 and 500 mg uscharin.

FIGURE 1

National Cancer Institute Developmental Therapeutics Program

Dose Response Curves

NSC: D-654033-O/1	SSPL: OCXW	Exp. ID: 9207SC89
Report Date: September 8, 1992	Test Date: July 20, 1992	



National Cancer Institute Developmental Therapeutics Program

In-Vitro Testing Results (PCT/GB 9 8 / 0 1 5 2 2

NSC: D- 654033 -O/1	Experiment ID: 9207SC89	Test Type: 8	Units: Molar
Report Date: September 8, 1992	Test Date: July 20, 1992	QNS:	MC:
COMI: Uscharin	Stain Reagent: Dual-Pass	SSPL: OCXW	

Panel/Cell Line	Time Zero	Ctrl	Log10 Concentration Mean Optical Densities -8.0	-7.0	-6.0	-5.0	-4.0	Percent Growth -8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.279	0.993	0.912	0.166	0.134	0.134	0.124	89	-41	-52	-52	-56	1.99E-08	4.66E-08	6.73E-07
HL-60(TB)	0.357	1.228	1.324	0.102	0.104	0.100	0.102	111	-71	-71	-72	-71	2.16E-08	4.06E-08	7.63E-08
K-562	0.120	0.823	0.904	0.132	0.085	0.104	0.111	111	4	-30	-13	-7	3.74E-08	1.33E-07	>1.00E-04
MOLT-4	0.498	1.377	1.463	0.194	0.163	0.151	0.337	90	-60	-67	-69	-31	1.83E-08	3.85E-08	
RPMI-8226	0.345	1.374	1.350	0.414	0.284	0.316	0.276	97	-24	-46	-42	-49	2.43E-08	6.34E-08	>1.00E-04
SR	0.348	1.430	1.279	0.138	0.127	0.094	0.150	84	-60	-64	-73	-57	1.73E-08	3.83E-08	8.48E-08
Non-Small Cell Lung Cancer															
A549/ATCC	0.381	1.657	1.595	0.133	0.076	0.109	0.098	95	-65	-80	-71	-74	1.91E-08	3.92E-08	8.04E-08
EKVX	1.134	1.726	1.790	0.617	0.244	0.352	0.162	111	-47	-79	-70	-86	3.43E-08	5.06E-08	1.26E-07
HOP-18															
HOP-62	0.464	1.702	1.699	0.208	0.035	0.018	0.014	100	-76	-96	-98	-98	1.92E-08	3.70E-08	7.12E-08
HOP-92	0.636	0.957	0.970	0.554	0.269	0.214	0.173	104	-13	-58	-66	-73	2.89E-08	7.75E-08	6.75E-07
NCI-H226	0.919	1.325	1.347	0.572	0.199	0.220	0.093	110	-38	-78	-76	-90	2.56E-08	5.57E-08	2.01E-07
NCI-H23	0.316	1.407	1.201	0.087	0.080	0.157	0.250	77	-83	-84	-70	-32	1.47E-08	3.02E-08	6.21E-08
NCI-H322M	0.564	1.460	1.519	0.620	0.461	0.349	0.273	104	6	-18	-38	-52	3.57E-08	1.79E-07	7.60E-05
NCI-H460	0.177	1.224	1.181	0.030	0.013	-0.002	0.018	96	-83	-93	-100	-90	1.80E-08	3.43E-08	6.52E-08
NCI-H522	0.476	0.763	0.729	0.130	0.044	0.068	0.098	88	-73	-91	-86	-80	1.73E-08	3.53E-08	7.23E-08
LXFL 529	0.456	1.485	1.493	0.054	0.018	0.012	0.015	101	-68	-96	-97	-97	1.86E-08	3.41E-08	6.27E-08
Small Cell Lung Cancer															
DMS 114	0.440	1.308	0.710	0.204	0.100	0.158	0.116	31	.	-77	-64	-74	<1.00E-08	3.74E-08	3.12E-07
DMS 273	0.256	1.331	1.342	-0.001	-0.012	0.013	0.016	101	-100	-100	-95	-94	1.79E-08	3.18E-08	5.64E-08
Colon Cancer															
COLO 205	0.277	1.284	1.213	0.300	0.067	0.188	0.091	93	2	-69	-32	-67	2.98E-08	1.08E-07	
DLD-1	0.153	0.846	0.844	0.035	0.026	0.012	0.030	97	-77	-83	-92	-80	1.86E-08	3.39E-08	6.96E-08
HCC-2998	0.306	0.817	0.908	0.336	0.022	0.004	0.010	110	6	-93	-99	-97	4.03E-08	1.13E-07	3.68E-07
HCT-116	0.238	1.376	1.265	0.094	0.016	0.031	0.069	90	-60	-93	-87	-71	1.85E-08	3.98E-08	8.53E-08
HCT-15	0.318	1.790	1.881	0.073	0.072	0.037	0.060	106	-77	-77	-88	-81	2.03E-08	3.81E-08	7.16E-08
HT29	0.248	1.271	1.342	0.221	0.031	0.046	0.038	107	-11	-80	-81	-84	3.03E-08	8.05E-08	3.70E-07
KM12															
KM20L2	0.364	1.047	1.034	0.152	0.012	0.008	0.007	101	-43	-95	-97	-97	2.27E-08	5.03E-08	1.38E-07
SW-620	0.229	1.324	1.299	0.179	0.077	0.134	0.133	98	-22	-66	-41	-42	2.91E-08	6.97E-08	
CNS Cancer															
SF-268	0.496	1.240	1.109	0.338	0.049	0.045	0.093	82	-32	-90	-90	-81	1.92E-08	5.27E-08	2.06E-07
SF-295	0.703	1.521	1.536	0.409	0.301	0.171	0.078	102	-42	-57	-76	-89	2.30E-08	5.12E-08	1.39E-07
SF-539	0.846	1.793	1.702	0.304	0.061	0.093	0.113	90	-64	-93	-89	-87	1.83E-08	3.85E-08	8.11E-08
SNB-19	0.856	1.894	1.910	1.028	0.434	0.536	0.337	102	16	-47	-30	-61	4.03E-08	1.81E-07	4.43E-05
SNB-75	0.564	0.864	0.611	0.572	0.305	0.414	0.380	82	3	-10	-27	-33	2.33E-08	1.60E-07	>1.00E-04
SNB-78	0.517	1.093	1.118	0.474	0.426	0.406	0.363	105	-13	-24	-27	-35	2.86E-08	7.50E-08	>1.00E-04
U251	0.269	1.179	1.224	0.062	0.016	0.006	0.018	103	-77	-94	-98	-93	2.00E-08	3.78E-08	7.11E-08
XF 498	0.469	0.713	0.710	0.162	0.042	0.014	0.015	99	-66	-91	-97	-97	1.98E-08	3.99E-08	8.04E-08
Melanoma															
LOX IMVI	0.236	1.363	1.346	0.016	-0.001	0.017	0.016	98	-94	-100	-94	-94	1.78E-08	3.23E-08	5.91E-08
MALME-3M	0.644	1.259	1.255	0.235	0.124	0.066	0.069	99	-64	.	-90	-89	2.01E-08	4.06E-08	8.26E-08
M14	0.333	1.167	1.160	0.240	0.038	0.035	0.004	99	-28	-89	-90	-99	2.44E-08	6.03E-08	2.31E-07
MDA-MB-435	0.284	1.126	1.124	0.386	0.094	0.144	0.146	100	12	-67	-49	-49	3.69E-08	1.42E-07	
SK-MEL-2	0.370	1.357	1.322	0.280	0.091	0.079	0.073	96	-51	-84	-86	-87	2.05E-08	4.30E-08	9.88E-08
SK-MEL-28	0.254	0.562	0.604	0.278	0.198	0.170	0.093	115	8	-22	-33	-64	4.03E-08	1.80E-07	3.56E-05
SK-MEL-5	0.445	1.805	1.896	0.249	0.200	0.179	0.134	99	-49	-59	-63	-72	2.16E-08	4.49E-08	1.36E-07
UACC-257	0.734	2.040	2.117	0.872	0.483	0.463	0.344	106	11	-34	-37	-53	3.86E-08	1.72E-07	6.40E-05
UACC-62	0.516	1.714	1.649	0.465	0.103	0.163	0.095	95	-10	-80	-68	-82	2.67E-08	6.04E-08	3.73E-07
Ovarian Cancer															
IGROV1	0.444	1.377	1.422	0.510	0.257	0.302	0.220	105	7	-42	-32	-28	3.63E-08	1.39E-07	>1.00E-04
OVCAR-3	0.654	1.189	1.238	0.280	0.324	0.298	0.237	109	-57	-51	-35	-61	2.27E-08	4.53E-08	9.03E-08
OVCAR-4	0.417	1.051	0.974	0.048	0.011	0.005	0.004	88	-88	-97	-99	-99	1.64E-08	3.13E-08	6.05E-08
OVCAR-5	0.344	0.848	0.852	0.043	0.017	-0.008	0.012	101	-88	-95	-100	-97	1.86E-08	3.42E-08	6.31E-08
OVCAR-8	0.615	1.784	1.799	0.530	0.092	0.043	0.206	101	-14	-85	-90	-67	2.79E-08	7.40E-08	3.22E-07
SK-OV-3	0.485	1.165	1.097	0.480	0.172	0.251	0.122	90	-1	-66	-48	.	2.75E-08	9.72E-08	
Renal Cancer															
786-0	0.274	1.093	1.062	0.027	0.011	0.006	0.027	96	-90	-96	-97	-90	1.77E-08	3.26E-08	6.09E-08
A498	0.418	1.360	1.346	0.672	0.718	0.544	0.341	98	34	13	-12	-45	5.46E-08	3.38E-08	>1.00E-04
ACHN	0.412	1.349	1.284	0.130	0.034	0.020	0.049	85	-68	-94	-95	-83	1.68E-08	3.57E-08	7.36E-08
CAKI-1															
RXF-393	0.836	1.266	1.203	0.613	0.391	0.499	0.868	84	-28	-54	-42	-22	2.02E-08	5.61E-08	
RXF-631															
SN12C	0.239	1.533	1.531	0.022	0.036	0.020	0.040	100	-91	-85	-92	-83	1.63E-08	3.34E-08	6.11E-08
TK-10	0.630	1.057	1.064	0.227	0.042	0.060	-0.088	102	.	-94	-91	-86	3.36E-08	1.10E-07	3.58E-07
UO-31	0.789	1.347	1.424	0.715	0.462	0.466	0.607	114	-9	-41	-41	-23	3.30E-08	8.40E-08	>1.00E-04

TABLE 1A

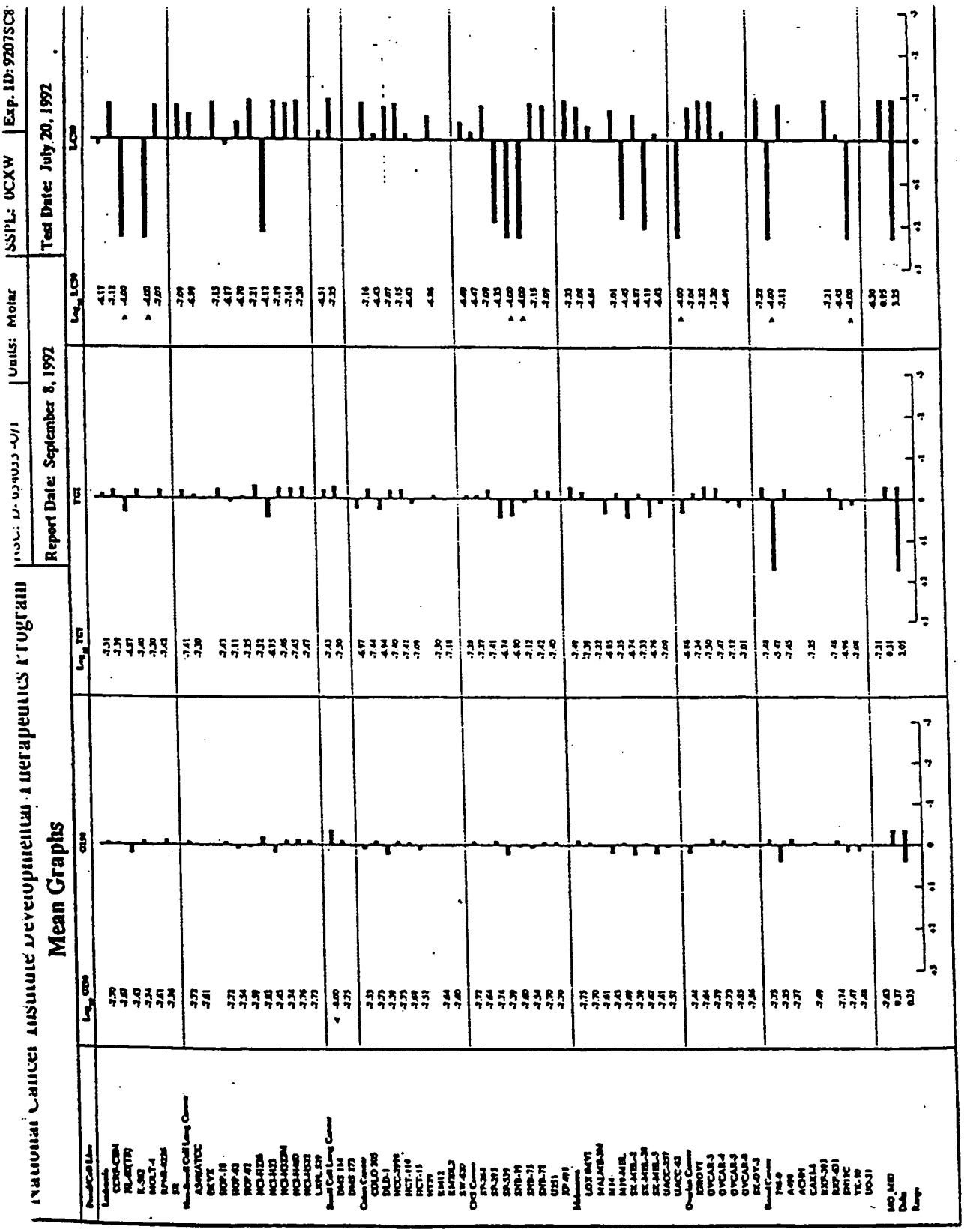
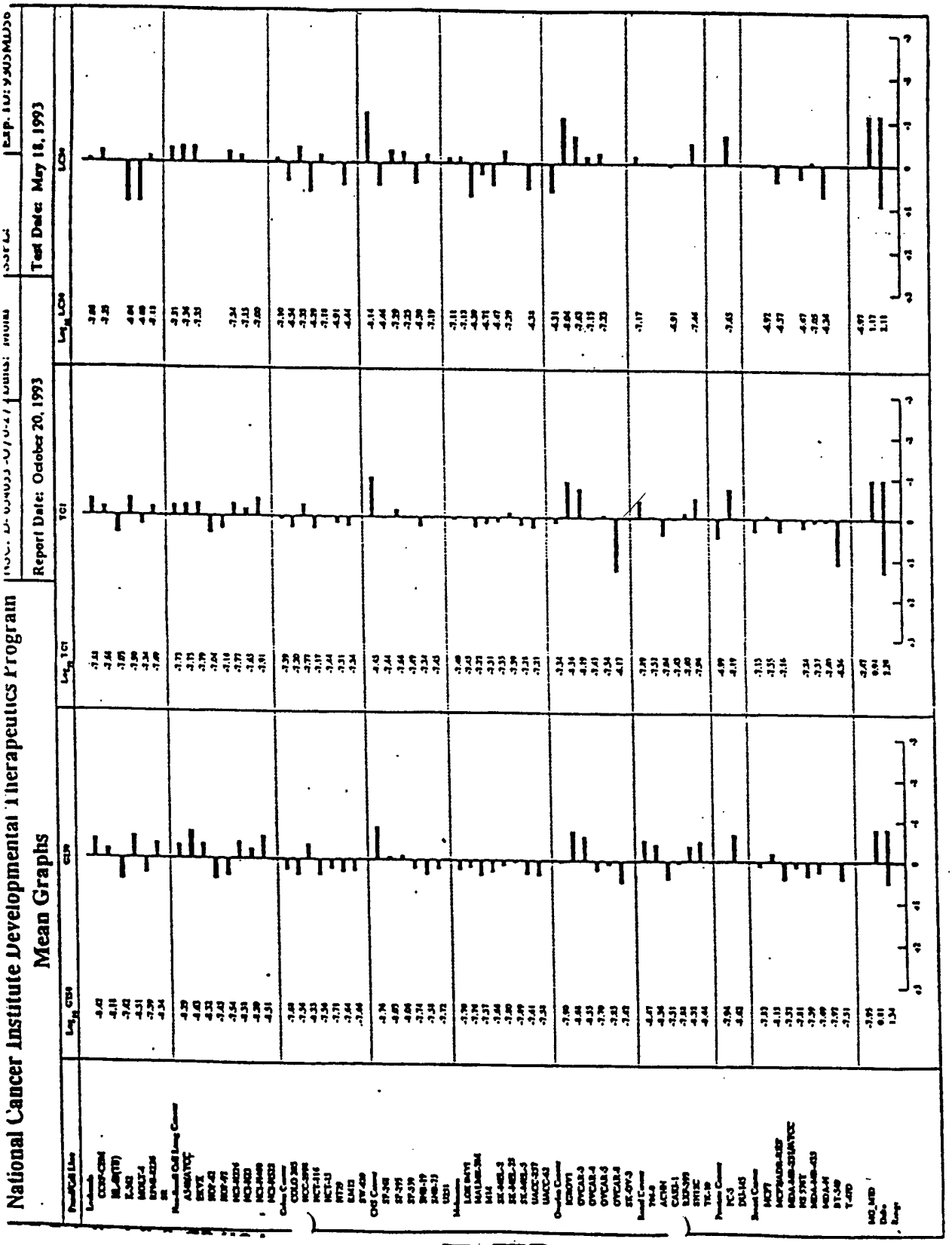

National Cancer Institute Developmental Therapeutics Program
Mean Graphs
Report Date: September 8, 1992
Test Date: July 20, 1992
Units: Molar
SSPL: UCXW
Exp. ID: 9207SC8

Table 1G


National Cancer Institute Developmental Therapeutics Program
Mean Graphs
Report Date: October 20, 1993
Test Date: May 18, 1993
Panel/Cell Line
Leukemia
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR
Non-Small Cell Lung Cancer
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522
Colon Cancer
COLO 205
HCC-2998
HCT-116
HCT-15
HT29
KM12
SW-620
CNS Cancer
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251
Melanoma
LOX IMVI
MALME-3M
M14
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62
Ovarian Cancer
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3
Renal Cancer
786-0
A498
ACHN
CAKI-1
RXF 393
SN12C
TK-10
UO-31
Prostate Cancer
PC-3
DU-145
Breast Cancer
MCF7
MDA-MB-231/ATCC
HS 578T
MDA-MB-435
BT-549
T-47D
MG_MID
Delta
Range
TABLE 2

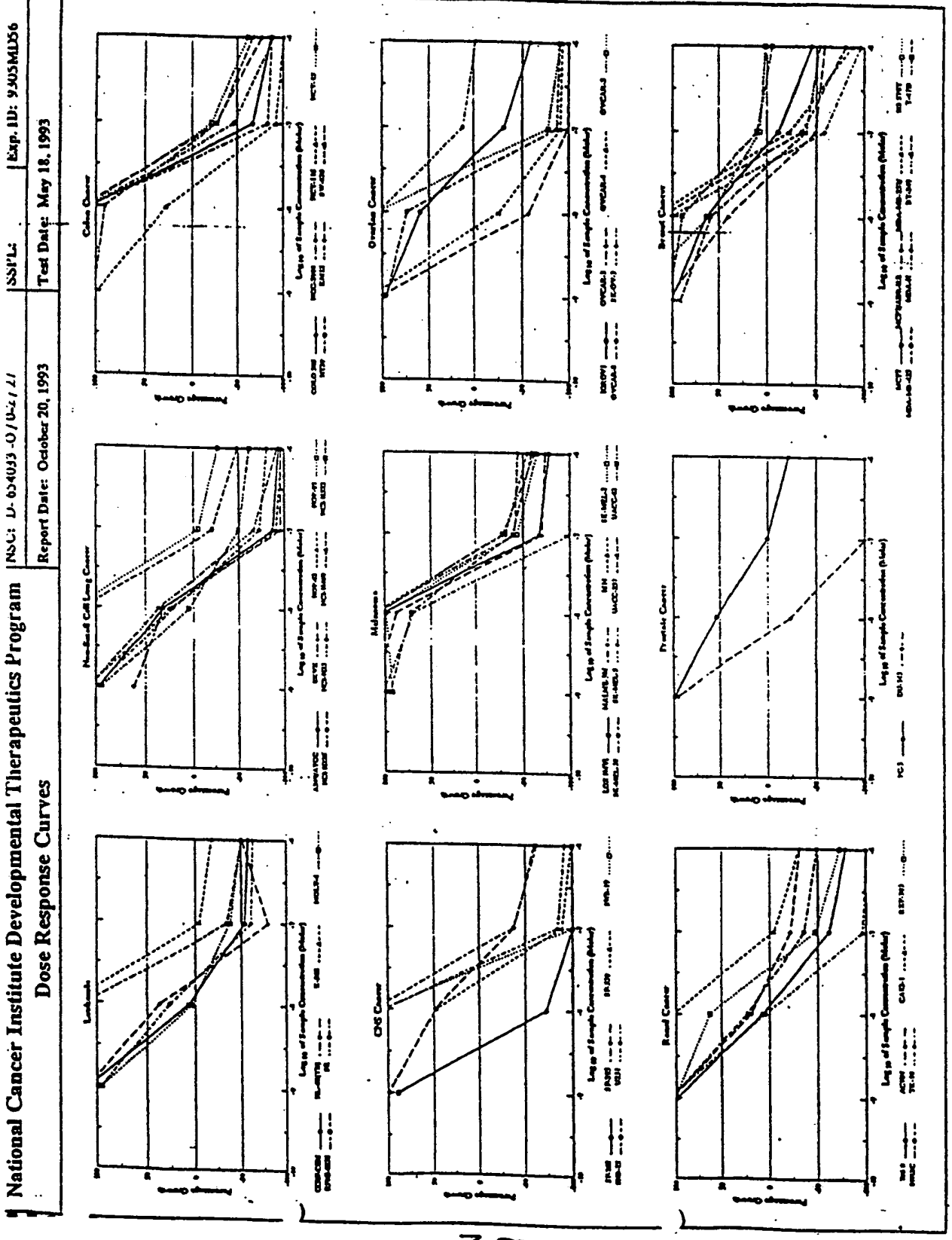

National Cancer Institute Developmental Therapeutics Program
Dose Response Curves
NSC: D-624033-0/D-624177
Report Date: October 20, 1993
SSPL:
Test Date: May 18, 1993
Exp. ID: 9303MD56
Colon Cancer
Ovarian Cancer
Breast Cancer
Non-Small Cell Lung Cancer
Melanoma
Prostate Cancer
Leukemia
CNS Cancer
Renal Cancer
Percent Growth
Log10 of Sample Concentration (Molar)

FIGURE 2

TABLE 3

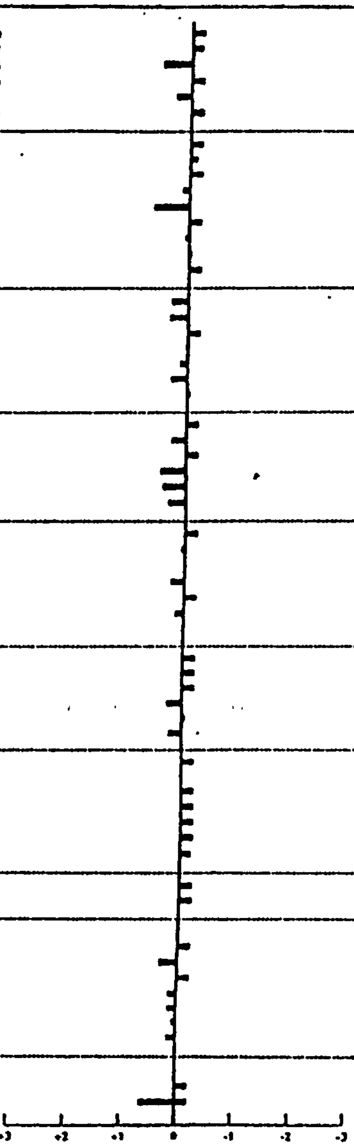
National Cancer Institute Developmental Therapeutics Program

Mean Graphs — USCHARIDIN

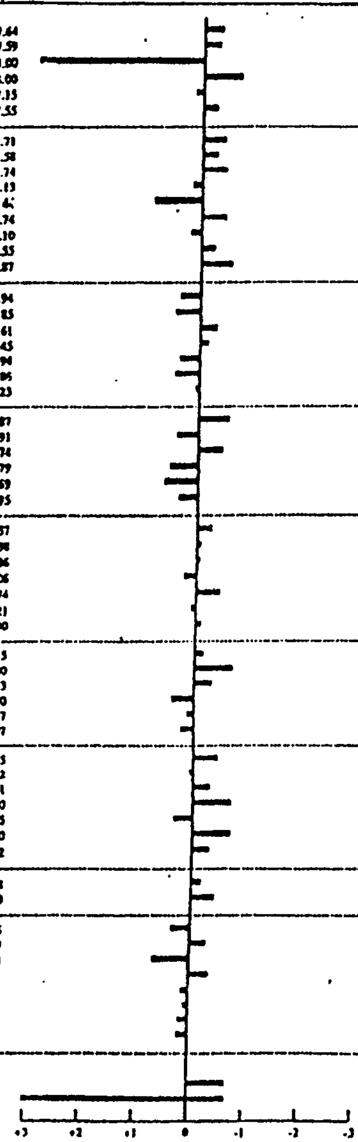
NSC: D- 659471 -Y/0-1/	Units: Molar	SSPL:	Exp. ID: 9302MD33
Report Date: March 8, 1993		Test Date: February 23, 1993	

Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	< -8.00	-7.64	> -4.00
HL-60(TB)	-7.57	-7.59	-7.22
K-562	-7.25	> -4.00	> -4.00
MOLT-4	< -8.00	< -8.00	-7.35
RPMI-8226	-7.51	-7.15	> -4.00
SR	< -8.00	-7.55	> -4.00
Non-Small Cell Lung Cancer			
A549/ATCC	< -8.00	-7.71	-7.29
EKVX	-7.91	-7.58	-7.25
HOP-62	< -8.00	-7.74	7.72
HOP-92	-7.65	-7.13	5.77
NCI-H226	-7.15	-6.46	> -4.00
NCI-H23	< -8.00	-7.74	-7.27
NCI-H322M	-7.71	-7.10	-6.39
NCI-H460	-7.83	-7.55	-7.26
NCI-H522	< -8.00	-7.87	-6.83
Colon Cancer			
COLO 205	-7.49	-6.94	-6.40
HCC-2998	-7.45	-6.85	-6.37
HCT-116	< -8.00	-7.61	-7.16
HCT-15	-7.76	-7.45	-7.14
HT29	-7.64	-6.94	-6.47
KM12	-7.49	-6.86	-6.36
SW-620	-7.84	-7.23	-6.44
CNS Cancer			
SF-268	< -8.00	-7.87	-7.43
SF-295	-7.52	-6.91	-6.41
SF-539	< -8.00	-7.74	-7.34
SNB-19	-7.32	-6.79	-6.39
SNB-75	-7.37	-6.69	> -4.00
U251	-7.47	-6.95	-6.48
Melanoma			
MALME-3M	< -8.00	-7.57	-7.07
M14	-7.71	-7.38	-7.04
SK-MEL-2	-7.76	-7.36	-6.88
SK-MEL-28	-7.54	-7.06	-6.38
SK-MEL-5	< -8.00	-7.74	-7.22
UACC-257	-7.64	-7.21	-6.65
UACC-62	-7.75	-7.40	-7.05
Ovarian Cancer			
IGROV1	< -8.00	-7.45	-6.51
OVCAR-3	< -8.00	< -8.00	-7.36
OVCAR-4	-8.00	-7.63	-7.25
OVCAR-5	-7.51	-6.90	-6.41
OVCAR-8	-7.84	-7.17	-6.57
SK-OV-3	-7.55	-7.07	-6.51
Renal Cancer			
786-0	< -8.00	-7.75	-7.17
A498	-7.76	-7.22	-6.29
ACHN	< -8.00	-7.61	> -4.00
RXF-393	< -8.00	< -8.00	-7.28
SN12C	< -8.00	-6.95	> -4.00
TK-10	< -8.00	< -8.00	-7.20
UO-31	-7.96	-7.62	-7.27
Prostate Cancer			
PC-3	< -8.00	-7.48	-6.87
DU-145	< -8.00	-7.73	-7.15
Breast Cancer			
MCF7	-7.78	-6.96	-6.03
MCF7/ADR-RES	< -8.00	-7.59	-7.13
MDA-MB-231/ATCC	-7.47	-6.63	-5.79
HS 578T	< -8.00	-7.66	> -4.00
MDA-MB-435	-7.63	-7.16	-6.52
MDA-N	-7.62	-7.21	-6.67
BT-549	-7.71	-7.12	-6.46
T-47D	-7.62	-7.10	> -4.00
MG_MID	-7.78	-7.30	-6.33
Delta	0.22	0.70	1.11
Range	0.85	4.00	3.43

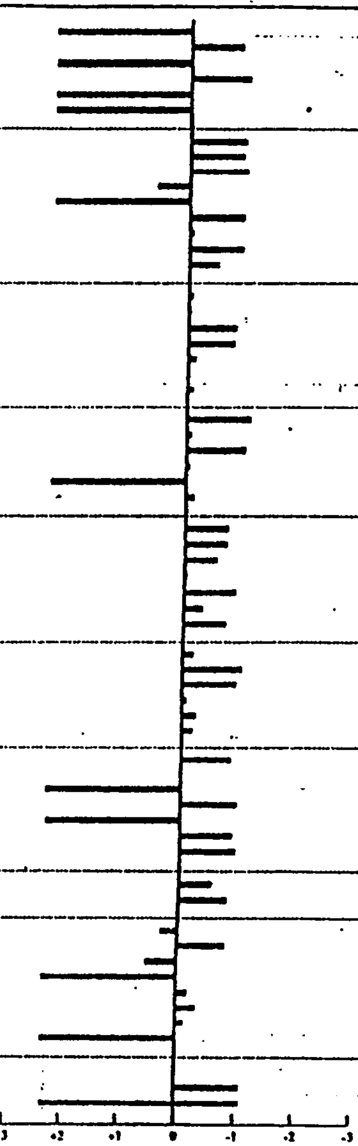
GI50



TGI



LC50



PCT/GB98/01522

National Cancer Institute Developmental Therapeutics Program

Mean Graphs- CALOTOXIN

NSC: D-659472-2/0-1/1	Units: Molar	SSPL:	Exp. ID: 9302MD33
Report Date: March 8, 1993		Test Date: February 23, 1993	

Panel/Cell Line	Log_{10} GI50	GI50	Log_{10} TGI	TGI	Log_{10} LC50	LC50
Leukemia						
CCRF-CEM	< -8.00		-7.51		> -4.00	
HL-60(TB)	-7.93		-7.52		-7.10	
K-562	-7.08		> -4.00		> -4.00	
MOLT-4	< -8.00		< -8.00		-5.28	
RPMI-8226	-7.57		-7.22		> -4.00	
SR	< -8.00				> -4.00	
Non-Small Cell Lung Cancer						
A549/ATCC	< -8.00		-7.63		-7.29	
EKVX	-7.95		-7.59		-7.22	
HOP-62	-7.95		-7.60		-7.25	
HOP-92	-7.69		-7.07		> -4.00	
NCI-H226	-7.28		-6.57		> -4.00	
NCI-H23	< -8.00		-7.78			
NCI-H322M	-6.86		-6.49		-6.13	
NCI-H460	-7.83		-7.54		-7.23	
NCI-H522	< -8.00		7.95		-6.92	
Colon Cancer						
COLO 205	-7.46		-6.95		-6.28	
HCC-2998	-7.52		-6.92		-6.38	
HCT-116	< -8.00		-7.52		-6.80	
HCT-15	-7.79		-7.47		-7.15	
HT29	-7.63		-6.94		-6.21	
KM12	-7.55		-6.94		-6.35	
SW-620	-7.82		-7.22		-6.45	
CNS Cancer						
SF-268	< -8.00		-7.72		-7.34	
SF-295	-7.50		-6.89		-6.40	
SF-539	< -8.00		-7.67		-7.28	
SNB-19	-7.42		-6.84		-6.41	
SNB-75	-7.18		-6.48		> -4.00	
U251	-7.50		-6.95		-6.48	
Melanoma						
MALME-3M	< -8.00		-7.51		-6.75	
M14	-7.73		-7.33		-6.65	
SK-MEL-2	-7.81		-7.38		-6.90	
SK-MEL-28	-7.48		-6.98		-6.27	
SK-MEL-5	< -8.00		-7.67		-7.19	
UACC-257	-7.63		-7.18		-6.60	
UACC-62	-7.69		-7.33		-7.02	
Ovarian Cancer						
IGROV1	< -8.00		-7.31		-6.36	
OVCAR-3	< -8.00		< -8.00		-7.20	
OVCAR-4	-7.96		-7.60		-7.23	
OVCAR-5	-7.53		-6.93		-6.42	
OVCAR-8	-6.81		-6.53		-6.25	
SK-OV-3	-7.55		-7.11		-6.53	
Renal Cancer						
786-0	< -8.00		-7.74		-7.08	
A498	< -8.00		-7.27		-6.32	
ACHN	< -8.00		-7.59		> -4.00	
RXF-393	< -8.00		< -8.00		-7.28	
SN12C	< -8.00		-7.00		> -4.00	
TK-10	< -8.00		< -8.00		-7.34	
UO-31	-7.93		-7.61		-7.29	
Prostate Cancer						
PC-3	< -8.00		-7.49		-6.85	
DU-145	< -8.00		-7.74		-7.25	
Breast Cancer						
MCF7	-6.94		-6.55		-6.16	
MCF7/ADR-RES	< -8.00		-7.54		-7.00	
MDA-MB-231/ATCC	-7.29		-6.34		-5.35	
HS 578T	< -8.00		-7.96		> -4.00	
MDA-MB-435	-7.67		-7.15		-6.38	
MDA-N	-7.60		-7.17		-6.62	
BT-549	-7.76		-7.20		-6.39	
T-47D	-7.65		-7.16		> -4.00	
SR 3183	-7.73		-7.25		-6.19	
Delta	0.27		0.75		1.17	
Range	1.19		4.00		3.36	

+3 +2 +1 0 -1 -2 -3

TABLE 4

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FIGURE 4

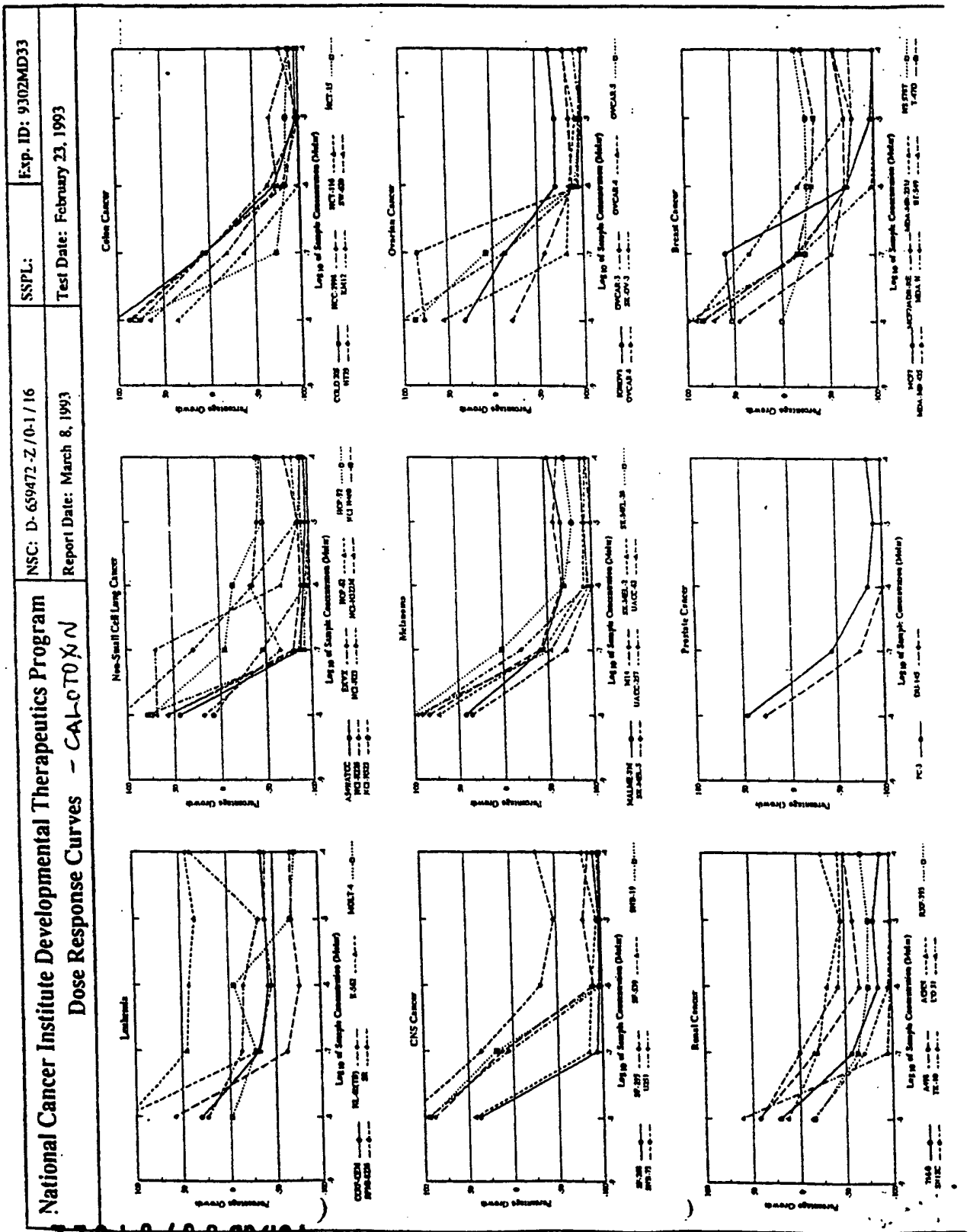
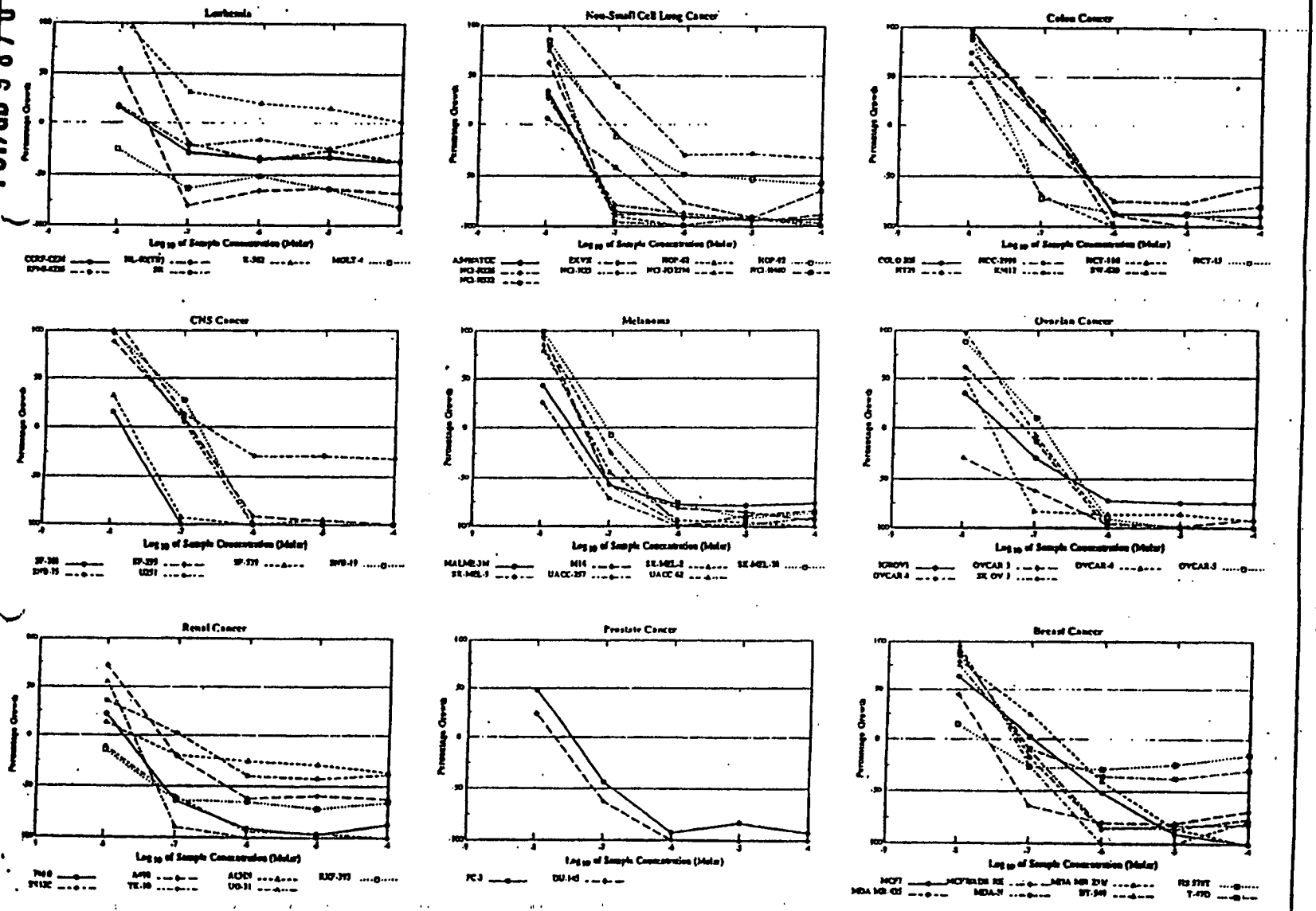

National Cancer Institute Developmental Therapeutics Program
Dose Response Curves - CAMPTOTHECIN
NSC: D- 659472 -Z /0-1 / 16
Report Date: March 8, 1993
SSPL:
Test Date: February 23, 1993
Exp. ID: 9302MD33
Colon Cancer
Ovarian Cancer
Breast Cancer
Non-Small Cell Lung Cancer
Melanoma
Prostate Cancer
Leukemia
CNS Cancer
Renal Cancer
Fractional Growth
Log 10 of Sample Concentration (Molar)

FIGURE 6/9

National Cancer Institute Developmental Therapeutics Program	NSC: D-659471-Y/0-1/15	SSPL:	Exp. ID: 9302MD33
Dose Response Curves – USCHARIDIN	Report Date: March 8, 1993	Test Date: February 23, 1993	



INTERNATIONAL SEARCH REPORT

Int ional Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/365

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.A. PARSONS: "Cat assay for the emetic action of digitalis and elated glycosides (digitoxin, digoxin, lanatoside C ouabain and calactin)" BR. J. PHARMACOL., vol. 42, no. 1, 1971, pages 143-152, XP002078318 see page 145 ---	1-8
P,X	F. KIUCHI ET AL.: "Cytotoxic priciples of a Bangladesh crude drug, akond mul (roots of Calotropis gigantea L.)" CHEM. PHARM. BULL., vol. 46, no. 3, 1998, pages 528-530, XP002078319 see the whole document ---	1-6
A	WO 92 09295 A (MRAK, M.,) 11 June 1992 ---	
	-/--	

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 September 1998	02/10/1998

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/[redacted]8/01522

C.(Continuation) DOCUMENTS CON[redacted]RED TO BE RELEVANT

Category *	Citation of document. with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	A.E.MUTLIB ET AL.: "In vivo and in vitro metabolism of gomphoside, a cardiotonic steroid with doubly-linked sugar." J. STEROID BIOCHEM., vol. 28, no. 1, 1987, pages 65-76, XP002078320	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]tional Application No
PCT/GB[illegible]/01522

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9209295 A	11-06-1992	CH 679012 A	13-12-1991
		AU 657283 B	09-03-1995
		AU 8902891 A	25-06-1992
		EP 0514508 A	25-11-1992

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(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES

(57) Abstract

The invention provides compositions comprising uscharin and the use of uscharin to combat cell proliferation for example in the treatment of cancer. Administration of uscharin may kill or reduce the growth rate of cancer cells and may also be of application in other medical conditions presenting symptoms of excessive or uncontrolled cell proliferation. The composition may be administered by any convenient route and formulated accordingly. The composition may be administered locally or generally and may be suitably dissolved and/or suspended in a pharmaceutically acceptable liquid carrier medium.

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PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES

This invention relates to a composition comprising the cardenolide glycoside uscharin.

Plants of the family *Asclepidaceae* are known to be extremely poisonous. Such plants have a history of use in folk medicines in those areas where they occur naturally, for example in South East Asia and Africa. Two of the best known representatives of the Asclepiadaceae are *Calotropis gigantea* and *Calotropis procera*. Extracts from *Calotropis procera* plants have traditionally been used as an abortifacient, for infanticide, for rheumatic pain and to produce a purgative.

The stems, flowers and leaves of plants from the family *Asclepiadaceae* (including *Calotropis gigantea* and *Calotropis procera)* are known to contain certain compounds known as cardenolides. In several species substantial amounts of cardenolides have been found to be concentrated in the latex (Roeske et al, in Biochemical Interactions Between Plants and Insects published in Volume 10 of Recent Advances in

Phytochemistry, Plenum Press, New York (ed. Wallace), Seiber et al, Phytochemistry 21:2343 (1982), Seiber et al, in Isopentoids in Plants, Academic Press (ed Nes, 1984) and Seiber et al, in J. Chem. Ecol. 6:321 (1980)). The natural production of cardenolides in Ascelopias curassavia has been reported by Groeneveld et al in Phytochemistry 29(11):3479-3486 (1990). Examples of cardenolide glycosides found in *C. procera* are voruscharin, uscharin, uscharidin, calotropin, calactin, calotoxin, and calotropagenin. Formula I shows the chemical structure of these cardenolides.

It has now been found that the cardenolide uscharin is particularly useful for medical purposes. Whilst uscharin has been isolated and its chemical structure determined, no utility for this compound has previously been reported.

The present invention thus provides a composition comprising uscharin, the analogues and salts thereof as active ingredient together with a pharmaceutically acceptable carrier or excipient.

Further, the present invention also provides the use of uscharin, the analogues and salts thereof for medical (including veterinary) purposes.

Previously, certain cardenolide glycosides such as calotropin and uzarigenin have been noted to have cytotoxic activity against primate tumour cells. Certain cardenolide glycosides from the *Asclepiadaceae* family share structural and pharmacological similarities with the *Digitalis* cardiac glycosides. Whilst we do not wish to be bound by theoretical considerations it is believed that the cytotoxicity of some cardenolide glycosides is related to the inhibition of the plasma membrane bound Na^+/K^+ ATPase (ie analogous to the manner in which *Digitalis* cardiac glycosides exert their toxic effects). However, it has also been shown that whilst some cardenolide glycosides are cytotoxic to cell cultures they have no <u>in vivo</u> tumour-inhibiting activity. This is true of calotropin and uzarigenin.

It has never previously been proposed that uscharin would be useful for medical applications. The inventors' results have shown that at 1mg/ml a primary

extract of *Calotropis gigantea* known as CGE-1 does have tumour inhibiting activity in rats (weighing about 200g) and does not lead to the death of the test animals.

Typically, the use of uscharin according to the present invention is to combat cell proliferation for example in the treatment of cancer. Thus administration of uscharin may kill or reduce the growth rate of cancer cells and may also be of application in other medical conditions presenting symptoms of excessive or uncontrolled cell proliferation.

The word "combat" is used herein to refer to treatment of an existing condition so as to alleviate or reverse the symptoms of the condition in an affected human or animal and to prevent such a condition in a healthy human or animal.

The composition according to the present invention may be administered by any convenient route and mention may be made of enteral, parenteral, topical administration and the composition will be formulated accordingly. Conveniently, the composition may be administered locally to the affected site, generally by means of injection. Thus the uscharin will be suitably dissolved and/or suspended in a pharmaceutically acceptable liquid carrier medium, which will generally be aqueous-based, for example an isotonic solution. Alternatively, the composition according to the invention may be taken orally.

Formulations for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers,

bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

The dose will depend on a number of factors known to the skilled physician including the severity of the conditions, the identity of the recipient; and also the efficacy and toxicity of the particular composition which is being administered. Generally doses in the range 0.1-100 mg/kg body weight may be used, particularly 1-10 mg/kg. The frequency of administration will vary depending on the rate of metabolism or excretion of the administered compound, but may be repeated daily, optionally as two or more sub-doses. Unit doses of 20 to 500 mg, preferably 100 to 400 mg may be used.

A single dosage may be given daily or smaller quantities or dosage units may be given at intervals throughout a 24 hour period, for example dosage units given 2, 3 or 4 times throughout the day.

Any type of cancer or condition involving cell proliferation may be treated by the present invention. Uscharin is especially useful for the treatment of

cancers such as leukaemia, non-small cell lung cancer, small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostrate cancer, and breast cancer. However the invention is not limited to treatment of these specific conditions since uscharin is believed to be of general effect.

Cancers where uscharin is particularly efficacious include ovarian cancer and skin cancer.

Uscharin may by produced by any convenient method, for example by chemical synthesis. Alternatively the uscharin may be conveniently extracted and purified from organisms (for example plants of the family *Asclepiadacaeae*) which produce uscharin naturally. It is also envisaged that uscharin may be manufactured using genetically engineered micro-organisms, plants or animals or may be made using cell-culture or other biotechnological techniques.

Further, the present invention also provides the use of a composition as described above for medical purposes, for example to combat conditions in which cell proliferation is undesirable (eg cancer).

In another aspect, the present invention provides the use of uscharin in the manufacture of a medicament. Generally such medicament would be of use to combat cancer and other conditions where cell proliferation is undesirable.

In a further aspect, the present invention provides a method of treatment of a human or non-human animal body, said method comprising administering to said body a composition as described above.

The present invention is now further described by means of the following, non-limiting Examples.

EXAMPLE 1

PREPARATION OF USCHARIN EXTRACT

(i) ISOLATION OF CGE-1

Leaves of *Calotropis gigantea* (500g) were Soxhlet extracted initially with petroleum ether (60-80), then ethyl acetate and finally methanol. The cell culture bioassays showed that the ethyl acetate fraction contained cytotoxic activity. The ethyl acetate extract was subjected to vacuum liquid chromatography (VLC) on silica gel 60H (Merck). Elution was initiated with petroleum ether (60-80) and proceeded with petroleum ether containing progressively greater amounts of ethyl acetate through to ethyl acetate only. Elution was then continued with ethyl acetate containing progressively greater amounts of methanol.

Samples of the fraction were collected and prepared for cytotoxicity testing by solubilisation in 0.1% Tween.

The greatest cytotoxic activity (ED_{50} <0.10µg/ml) was found in the 70-80% ethyl acetate in petroleum ether fractions. The cytotoxic compound CGE-1 (72.0 mg) (ED_{50}< 0.09µg/ml) was isolated as a white semi-crystalline precipitate from this fraction.

(ii) ISOLATION OF CGE-2

Another less cytotoxic compound, CGE-2 (101.0mg) (ED_{50} <8.0µg/ml) was isolated from the 100% ethyl acetate

fraction as a semi-crystalline precipitate.

(iii) PROPERTIES OF CGE-1

White powder, found 587.2511, $C_{31}H_{41}NO_8S$ requires 587,2553. $[\alpha]_0$ + 10.0° (c.0.1,CH_3OH_4) IR V_{max} CM^{-1}: 3465, 2960, 2920, 2840, 2720, 1735, 1730, 1705, 1625, 1540, 1160, 1110, 1060, 1040. EIMS m/z (rel. int.) 587 [M+] (4.0), 233 (14.9), 215 (8.6), 187 (9.8), 183

ACTIVITY OF CGE-1

At a concentration of 1 mg/ml, CGE-1 has a tumor inhibiting activity in rats weighing approximately 200g and does not lead to the death of the rat.

CGE-1 was found to contain Uscharin.

EXAMPLE 2

Isolation of Uscharin from *Calotropis Gigantea* leaves.

EXTRACTION

The plant material was minced to a fine powder in a bench grinder. The powder was extracted in a Soxhlet with petroleum ether (60-80) and the ethyl acetate, until exhaustion. The ethyl acetate fraction was concentrated to dryness using a rotary evaporator.

FRACTIONATION

Vacuum Liquid Chromatography was used for the initial fractionation of the crude extract Silica gel 60H

(Merck) was packed in a scintered funnel under vacuum to give a compact column. The crude extract, adsorbed in silica, was applied to the column. Elution was initiated with petroleum ether and proceeded with petroleum ether containing progressively greater amounts of ethyl acetate than with ethyl acetate through to methanol. The fractions were concentrated using a rotary evaporator. 10 mg of each fraction were prepared for cytotoxicity testing (see MTT assay for method) by solubilisation in DMSO. The fraction containing the greatest cytotoxic activity was subjected to a sephadex column to remove any remaining chlorophyll.

SEPHADEX COLUMN

The fraction was dissolved in a minimum volume of chloroform and applied to a column containing lipophilic sephadex LH-20 (Sigma) which had been packed in chloroform. Elution was with chloroform, chloroform with methanol and methanol. As before fraction were dried and tested for activity. The fraction with the greatest activity was further fractionated with a silica gel column.

SILICA GEL COLUMN

The fraction was dissolved in a minimum volume of chloroform and applied to a column containing silica gel (packed in chloroform). Elution was with chloroform, chloroform with methanol and methanol. This column yielded a fraction of almost pure uscharin. The pure compound was obtained from this fraction by preparative TLC.

PREPARATIVE TLC

The fraction was spotted onto glass silica gel plates. The plates were run in ethyl acetate and methanol (97:3). The silica was scratched from the plate and the uscharin eluted with ethyl acetate.

Once the compound had been isolated, its identity was confirmed by spectroscopic techniques.

EXAMPLE 3

CYTOTOXICITY BIOASSAY OF USCHARIN

Cytotoxicity bioassays were performed. The cell line used was a human ovarian small cell carcinoma SCC Wm 1(151) which was grown as a monolayer in Dulbecco's Modified Eagles Medium (Gibco) supplemented with 5% foetal calf serum (v/v), sodium pyruvate (1mM), penicillin (50IU/ml) and streptomycin (50µg/ml). Cultures were maintained in a humidified atmosphere of 5% CO_2/95% air at 37°.

Single cell suspensions were obtained by trypsinisation of the monolayer cultures and an equal number of cells (10^3-10^4 depending on the cell line) was inoculated into each 33mm² well of a 96 well plate in 190µl of culture medium. The plates were incubated for 24 hours to allow cells to adhere. At this point 10µl of an appropriate concentration of plant extract or control solvent was added to each well. The cells were exposed to the drug for 3 days after which the medium was removed, the monolayers washed with PBS and fresh medium added. This was repeated 24 hours later. Following a further 24 hours incubation 100µg (50µl of

2mg/ml in PBS) MTT (3-(4,5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was added to each well and the cells were incubated at 37°C for 4 hours. Plates were then processed using a modified version (Carmichael et al, 1987) of the assay first described by Mossman, T.(1983), where DMSO was used in preference to acid isopropanol to solubilise the formazan crystals. The contents of each well were mixed and the plate was read immediately at 540nm on a Flow Titertek Multiscan MCC/340 Mk 11 plate reader. Cells were set up in parallel at two densities, 10^3 and 2×10^3 cells/well, and the results from an assay were discarded if the ratio of the OD readings of the two densities was greater than 2.25:1 or less than 1.75:1.

The results obtained were as shown in Fig. 1

EXAMPLE 4

IN VITRO SCREENING OF USCHARIN

Uscharin was obtained as in Example 2 and was subjected to in vitro cell screening at the National Cancer Institute (NCI), USA in respect of a panel of cancel cell types organised into subpanels representing leukemia, lung cancers, colon cancer, cancer of the central nervous system, melanoma, ovarian cancer, renal cancer, and in some cases prostate cancer and breast cancer also.

The standard NCI methodology which was employed is described in Michael R Boyd, Principles and Practices of Oncology, Vol. 3, No. 10 (Oct. 1989) and Monks A. et al., Journal of the National Cancer Institute, Vol. 83, No. 11, (5th June, 1991).

The results of two separate screening experiements carried out using uscharin are given in Tables 1 and 2.

The data are derived from Dose-Response Curves and two typical curves for leukemia and colon cancer are given for illustrative purposes in Figures 1 and 2 attached hereto.

The Dose-Response Curve is created by plotting the Calculated Percent Growth (PG) of each cell line against the $\log_{(10)}$ of the corresponding drug concentration. The cell line curves are grouped by cell type, or subpanel. Mean $\text{Log}_{(10)}$ concentrations for all cell lines tested are calculated at three points: where the test compound achieved 50% inhibition of cell growth (GI_{50}), where the test compound achieved 0% cell growth or total growth inhibition (TGI), and where the test compound achiveved 50% cell kill or 50% lethal concentration (LC_{50}). Reference lines are shown at the percent growth values of +50 (GI_{50}), 0 (TGI) and -50 (LC_{50}).

Percentage Growth (PG) - of the compound on a cell line is currently calculated according to one of the following expressions:

If (Mean OD(test) - Mean OD(tzero) >= 0, then

PG = 100 x (Mean OD(test) - Mean OD(tzero)/(mean OD(ctrl) - Mean OD(tzero)

If (Mean OD(test - Mean OD(tzero) < 0, then PG = 100 x (Mean OD(test) - Mean OD(tzero)/Mean OD(tzero)

Where:

Mean OD (tzero) = The average of optical density measurements of SRB-derived colour just before exposure of cells to the test compound.

Mean OD (test) = The average of optical density measurements of SRB-derived colour after 48 hours with no exposure of cells to the test compound.

Mean OD (ctrl) = The average of optical density measurements of SRB-derived colour after 48 hours with no exposure of cells to the test compound.

It is clear from the results given in Tables 1 and 2 that uscharin has an inhibitory effect on the growth of a wide variety of cancer cell lines in vitro.

EXAMPLE 5

IN VITRO SCREENING OF USCHARIDIN

Uscharidin was also subjected to in vitro cell screening in the manner described in Example 4. Results are given in Table 3 and Figure 3, and these show that Uscharidin also exerts an inhibitory effect on a variety of cancer cell lines in vitro.

EXAMPLE 6

IN VITRO SCREENING OF CALOTOXIN

Calotoxin was also subjected to in vitro cell screening in the manner described in Example 4. Results are given in Table 4 and Figure 4, which show that calotoxin also exerts an inhibitory effect on a variety of cancer cell lines in vitro.

EXAMPLE 7

IN VITRO EXPERIEMENT WITH USCHARIN IN NUDE MICE

The SCCI cells (human tumour cell line) where grown (1 x 10^5/ml seeding density) in 25 ml RPMI 1640 (10% foetal calf serum, 5% glutamine) in 75 cm^2 tissue culture flasks. The cells were harvested at log growth phase (5 days approximately) and washed once in saline before injection into the mice.

The "nude" mice (BALB/c nude) are reared and contained within a sealed isolator. The mice were injected with 1 x 10^7 cells subcut on the back, right hand side near the shoulder blades. After 7 days the mice were split randomly into the study groups (10-15 animals per group). Each was then treated with a different regime, the variable being time between injections and dose of drug at each injection, control groups were also included in the overall plan of the experiement.

During the trial a daily check was made on the animals and any animal removed if the tumour size became too large (>5-7% total body weight) or if the animal is showing signs of distress. Additional to this the

tumour should be assessed every 3-4 days by an independent observer and the result recorded. Once an animal is removed from the study the tumour size, volume and weight was determined and the tumour stored for further cytological study. The reason for the animals removal from the study was also recorded, if this was not due to tumour size. The results are shown in the following tables.

Using nude mice injected with 10^7 SCC-1 cells injected on day 0 and drug treatment started on day 9.

GROUP NO. 1

0.1 mg CGE-1/ Animal/ 5 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	27	4356.4	1.7492	64.8	22.41	1
B	55	-	NONE	-	-	5
C	30	4141.3	2.5658	85.5	45.28	1
D	30	299.8	1.8196	60.7	52.24	1
E	37	2752.8	1.5783	42.7	33.37	1
F	55	-	NONE	-	-	5
G	55	-	NONE	-	-	5
H	55	-	NONE	-	-	5
I	33	3414.9	1.8805	57.0	28.69	1
J	55	-	NONE	-	-	5
K	37	828.9	0.6773	18.3	8.19	2
L	27	2223.8	1.6854	62.4	48.92	1
M	27	1556.2	0.7728	28.6	5.45	1
N	27	3457.9	1.9394	71.8	52.94	1
O	55	-	NONE	-	-	5
MEAN		2559.11	1.6298	54.64	33.05	
S.D.		1437.34	0.5844	21.20	18.29	

GROUP NO. 2

0.1 mg CGE-1/ Animal/ 10 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	27	2993.1	2.0570	76.2	49.92	1
B	55	-	NONE	-	-	5
C	55	-	NONE	-	-	5
D	55	-	NONE	-	-	5
E	55	664.8	0.4333	7.9	17.91	5
F	55	3148.8	2.0378	37.1	16.96	5
G	55	134.4	0.1285	2.3	8.17	5
H	55	-	NONE	-	-	5
I	55	-	NONE	-	-	5
J	55	-	NONE	-	-	5
K	55	-	NONE	-	-	5
L	55	-	NONE	-	-	5
M	26	2025.9	1.3238	50.9	6.90	3
N	30	1548.8	1.2677	42.3	10.79	1
O	30	544.1	0.3827	12.8	25.29	4
MEAN		1579.99	1.0901	32.79	19.42	
S.D.		1201.27	0.7933	26.68	14.9	

GROUP NO. 3

0.5 mg CGE-1/ Animal/ 5 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	55	-	NONE	-	-	5
B	55	219.6	0.2082	3.8	18.18	5
C	55	-	NONE	-	-	5
D	19	1494.7	1.1889	62.6	2.33	3
	19	203.2	0.0948	5.0	-	
E	19	-	NONE	-	-	3
F	23	3912.0	2.5341	110.2	13.13	1
G	28	4463.2	2.5717	91.8	23.42	1
H	37	-	NONE	-	-	2
I	28	1666.5	1.0930	39.0	12.96	1
J	19	23.7	0.0038	0.2	-	3
K	33	1457.9	1.2546	38.0	19.22	1
L	29	1532.5	0.8926	30.8	12.49	1
M	29	2972.3	1.6348	56.4	17.79	1
N	37	537.9	0.4997	13.5	9.70	2
O	37	-	NONE	-	-	2
MEAN		1848.36	1.1976	45.12	14.36	
S.D.		1504.32	0.8738	36.61	6.18	

GROUP NO. 4

0.5 mg CGE-1/ Animal/ 10 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	28	1482.1	1.1211	40.0	28.48	1
B	27	3499.1	2.5087	92.9	32.54	1
C	42	1930.3	1.4088	33.5	13.58	1
D	42	2177.3	1.5067	35.9	17.14	1
E	55	-	NONE	-	-	5
F	27	6882.3	3.1626	117.1	42.37	1
G	33	760.9	0.7467	22.6	50.31	1
H	55	-	NONE	-	-	5
I	55	-	NONE	-	-	5
J	55	-	NONE	-	-	5
K	55	64.5	0.1127	2.0	17.78	5
L	29	-	NONE	-	-	2
M	55	-	NONE	-	-	5
N	23	4929.6	2.6126	113.6	37.52	1
O	55	-	NONE	-	-	5
MEAN		2715.76	1.6475	57.2	29.97	
S.D.		2272.64	1.0344	44.08	13.18	

GROUP NO. 5

CONTROL (0.1 ml Saline/ Animal/ 5 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm^3)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	55	-	NONE	-	-	5
B	55	-	NONE	-	-	5
C	55	-	NONE	-	-	5
D	55	-	NONE	-	-	5
E	23	4570.9	2.4227	105.3	35.2	1
F	50	3138.3	1.9475	39.0	4.43	1
G	55	-	NONE	-	-	5
H	55	-	NONE	-	-	5
I	3	-	NONE	-	-	3
J	23	5493.0	3.1602	137.4	59.07	1
K	28	2500.7	1.8958	67.7	6.68	1
L	28	3246.9	1.9716	70.4	31.86	1
M	55	-	NONE	-	-	5
N	28	4120.3	2.2965	82.0	46.07	1
O	55	-	NONE	-	-	5
MEAN		3845.02	2.2707	83.63	30.55	
S.D.		1093.88	0.4797	34.01	21.59	

1 NOTES:-
2 REASONS:
3
4 (1) Removed due to tumour size.
5 (2) Removed due to another illness.
6 (3) Found dead in cage.
7 (4) Removed because the tumour was about to rupture.
8 (5) Removed at end of the experiment.
9

TABLE 5

Table 5 gives a summary of the results.

	Tumour Growth (mg/day)	% Necrosis*	% Mortality at 40 days
Group 1 (0.1mg/5 days)	54.6 ± 21.1	33.1 ± 18.3	84
Group 2 (0.1mg/10 days)	32.8 ± 26.7	19.4 ± 14.9	55
Group 3 (0.5mg/5 days)	45.1 ± 36.6	14.4 ± 6.2	90
Group 4 (0.5mg/10 days)	57.2 ± 44.1	30.0 ± 13.2	62
Control	83.6 ± 34.0	30.6 ± 21.6	100

* from histological examination
Values are means ±SD, n=15

From these results it can be seen that a reduction in percentage mortallity due to the cancer cells of up to 45% can be achieved by administration of the compound of the invention (Uscharin).

CLAIMS

1. A composition comprising uscharin or analogues or salts thereof as active ingredient together with a pharmaceutically acceptable carrier or excipient.

2. The use of uscharin, analogues or salts thereof for medical (including veterinary) purposes.

3. The use of uscharin as claimed in the preparation of a medicament.

4. A composition as claimed in Claim 1 or 2 wherein the uscharin is suspended or dissolved in an acceptable liquid carrier medium.

5. A composition as claimed in Claim 4 wherein the carrier medium is aqueous based.

6. A use as claimed in Claims 2 or 3 wherein 0.1-100 uscharin per kg body weight is used.

7. A method of treatment of a human or non-human animal body, said method comprising administering to said body a composition comprising uscharin.

8. A method as claimed in Claim 7 wherein a unit dose of composition comprises between 20 and 500 mg uscharin.

	Log10 Concentration						
	Time		Mean Optical Densitites				
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0
Leukemia							
CCRF-CEM	0.279	0.993	0.912	0.166	0.134	0.134	0.124
HL-60(TB)	0.357	1.228	1.324	0.102	0.104	0.100	0.102
K-562	0.120	0.825	0.904	0.152	0.085	0.104	0.111
MOLT-4	0.490	1.577	1.463	0.194	0.163	0.151	0.337
RPMI-8226	0.545	1.374	1.350	0.414	0.284	0.316	0.276
SR	0.348	1.450	1.279	0.138	0.127	0.094	0.150
Non-Small Cell Lung Cancer							
A549/ATCC	0.381	1.657	1.595	0.133	0.076	0.109	0.098
EKVX	1.154	1.728	1.790	0.617	0.244	0.352	0.162
HOP-18							
HOP-62	0.864	1.702	1.699	0.208	0.035	0.018	0.014
HOP-92	0.636	0.957	0.970	0.554	0.269	0.214	0.173
NCI-H226	0.919	1.325	1.367	0.572	0.199	0.220	0.093
NCI-H23	0.516	1.407	1.201	0.087	0.080	0.157	0.250
NCI-H322M	0.564	1.480	1.519	0.620	0.461	0.349	0.273
NCI-H460	0.177	1.224	1.181	0.030	0.013	-0.002	0.018
NCI-H522	0.476	0.763	0.729	0.130	0.044	0.068	0.098
LXFL 529	0.456	1.485	1.493	0.054	0.018	0.012	0.015
Small Cell Lung Cancer							
DMS 114	0.440	1.308	0.710	0.204	0.100	0.158	0.116
DMS 273	0.256	1.331	1.342	-0.001	-0.012	0.013	0.016
Colon Cancer							
COLO 205	0.277	1.284	1.215	0.300	0.087	0.186	0.091
DLD-1	0.153	0.866	0.844	0.035	0.026	0.012	0.030
HCC-2998	0.306	0.817	0.908	0.336	0.022	0.004	0.010
HCT-116	0.235	1.376	1.265	0.094	0.016	0.031	0.069
HCT-15	0.318	1.790	1.881	0.075	0.072	0.037	0.060
HT29	0.248	1.271	1.342	0.221	0.051	0.046	0.038
KM12							
KM20L2	0.264	1.047	1.054	0.152	0.012	0.008	0.007
SW-620	0.229	1.324	1.299	0.179	0.074	0.134	0.133
CNS Cancer							
SF-268	0.496	1.240	1.109	0.338	0.049	0.049	0.093
SF-295	0.703	1.521	1.536	0.409	0.301	0.171	0.078
SF-539	0.846	1.793	1.702	0.304	0.061	0.093	0.113
SNB-19	0.856	1.894	1.910	1.028	0.454	0.598	0.337
SNB-75	0.564	0.864	0.811	0.572	0.505	0.414	0.380
SNB-78	0.557	1.093	1.118	0.474	0.426	0.405	0.363
U251	0.269	1.179	1.224	0.062	0.016	0.006	0.018
XF 498	0.469	0.713	0.710	0.162	0.042	0.014	0.015

Fig. 1a

Log10 Concentration

Panel/Cell Line	Time		Mean Optical Densitites				
	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0
Melanoma							
LOX IMVI	0.256	1.365	1.346	0.016	-0.001	0.017	0.016
MALME-3M	0.644	1.259	1.255	0.235	0.124	0.066	0.069
M14	0.333	1.167	1.160	0.240	0.038	0.035	0.004
M19-MEL	0.284	1.126	1.124	0.386	0.094	0.144	0.146
SK-MEL-2	0.570	1.357	1.322	0.280	0.091	0.078	0.075
SK-MEL-28	0.254	0.562	0.608	0.278	0.198	0.170	0.093
SK-MEL-5	0.485	1.905	1.896	0.249	0.200	0.179	0.134
UACC-257	0.734	2.040	2.117	0.872	0.483	0.463	0.344
UACC-62	0.516	1.714	1.649	0.465	0.103	0.163	0.095
Ovarian Cancer							
IGROV1	0.444	1.377	1.422	0.510	0.257	0.302	0.320
OVCAR-3	0.654	1.189	1.238	0.280	0.324	0.296	0.257
OVCAR-4	0.417	1.051	0.974	0.048	0.011	0.005	0.004
OVCAR-5	0.346	0.848	0.852	0.043	0.017	-0.008	0.012
OVCAR-8	0.615	1.784	1.799	0.530	0.092	0.065	0.206
SK-OV-3	0.485	1.165	1.097	0.480	0.172	0.251	0.122
Renal Cancer							
786-0	0.274	1.093	1.062	0.027	0.011	0.008	0.027
A498	0.618	1.360	1.348	0.872	0.718	0.544	0.341
ACHN	0.412	1.349	1.204	0.130	0.024	0.020	0.069
CAKI-1							
RXF-393	0.856	1.266	1.203	0.613	0.391	0.499	0.668
RXF-631							
SN12C	0.239	1.533	1.531	0.022	0.036	0.020	0.040
TK-10	0.650	1.057	1.064	0.227	0.042	0.060	0.088
UO-31	0.789	1.347	1.424	0.715	0.462	0.466	0.607

Fig. 1b

	Log10 Concentration							
	Percent Growth							
Panel/Cell Line	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia								
CCRF-CEM	89	-41	-52	-52	-56	1.99E-08	4.86E-08	6.73E-07
HL-60(TB)	111	-71	-71	-72	-71	2.16E-08	4.06E-08	7.63E-08
K-562	111	4	-30	-13	-7	3.74E-08	1.35E-07	>1.00E-04
MOLT-4	90	-60	-67	-69	-31	1.83E-08	3.95E-08	
RPMI-8226	97	-24	-48	-42	-49	2.45E-08	6.34E-08	>1.00E-04
SR	84	-60	-64	-73	-57	1.73E-08	3.83E-08	8.48E-08
Non-Small Cell Lung Cancer								
A549/ATCC	95	-65	-80	-71	-74	1.91E-08	3.92E-08	8.04E-08
EKVX	111	-47	-79	-70	-86	2.43E-08	5.06E-08	1.28E-07
HOP-18								
HOP-62	100	-76	-96	-98	-98	1.92E-08	3.70E-08	7.12E-08
HOP-92	104	-13	-58	-66	-73	2.89E-08	7.75E-08	6.75E-07
NCI-H226	110	-38	-78	-76	-90	2.56E-08	5.57E-08	2.01E-07
NCI-H23	77	-83	-84	-70	-52	1.47E-08	3.02E-08	6.21E-08
NCI-H322M	104	6	-18	-38	-52	3.57E-08	1.79E-07	7.60E-05
NCI-H460	96	-83	-93	-100	-90	1.80E-08	3.43E-08	6.52E-08
NCI-H522	88	-73	-91	-86	-80	1.73E-08	3.53E-08	7.23E-08
LXFL 529	101	-88	-96	-97	-97	1.86E-08	3.41E-08	6.27E-08
Small Cell Lung Cancer								
DMS 114	31	.	-77	-64	-74	<1.00E-08	3.74E-08	3.12E-07
DMS 273	101	-100	-100	-95	-94	1.79E-08	3.18E-08	5.64E-08
Colon Cancer								
COLO 205	93	2	-69	-32	-67	2.98E-08	1.08E-07	.
DLD-1	97	-77	-83	-92	-80	1.86E-08	3.59E-08	6.96E-08
HCC-2998	118	6	-93	-99	-97	4.03E-08	1.15E-07	3.68E-07
HCT-116	90	-60	-93	-87	-71	1.85E-08	3.98E-08	8.55E-08
HCT-15	106	-77	-77	-88	-81	2.03E-08	3.81E-08	7.16E-08

Fig. 1c

Log10 Concentration

Panel/Cell Line	Percent Growth -8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Colon Cancer								
HT29	107	-11	-80	-81	-84	3.03E-08	8.05E-08	3.70E-07
KM12	101	-43	-95	-97	-97	2.27E-08	5.05E-08	1.38E-07
KM20L2	98	-22	-68	-41	-42	2.51E-08	6.57E-08	.
SW-620								
CNS Cancer								
SF-268	82	-32	-90	-90	-81	1.92E-08	5.27E-08	2.06E-07
SF-295	102	-42	-57	-76	-89	2.30E-08	5.12E-08	3.39E-07
SF-539	90	-64	-93	-89	-87	1.83E-08	3.85E-08	8.11E-08
SNB-19	102	16	-47	-30	-61	4.03E-08	1.81E-07	4.43E-05
SNB-75	82	3	-10	-27	-33	2.53E-08	1.60E-07	>1.00E-04
SNB-78	105	-15	-24	-27	-35	2.86E-08	7.50E-08	>1.00E-04
U251	105	-77	-94	-96	-93	2.00E-08	3.78E-08	7.11E-08
XF 498	99	-66	-91	-97	-97	1.98E-08	3.99E-08	8.04E-08
Melanoma								
LOX IMVI	98	-94	-100	-94	-94	1.78E-08	3.25E-08	5.91E-08
MALME-3M	99	-64	.	-90	-89	2.01E-08	4.08E-08	8.26E-08
M14	99	-28	-89	-90	-99	2.44E-08	6.03E-08	2.31E-07
M19-MEL	100	12	-67	-49	-49	3.69E-08	1.42E-07	.
SK-MEL-2	96	-51	-84	-86	-87	2.05E-08	4.50E-08	9.88E-08
SK-MEL-28	115	8	-22	-33	-64	4.03E-08	1.80E-07	3.56E-05
SK-MEL-5	99	-49	-59	-63	-72	2.16E-08	4.69E-06	1.36E-07
UACC-257	106	11	-34	-37	-53	3.86E-08	1.72E-07	6.40E-05
UACC-62	95	-10	-80	-68	-82	2.67E-08	8.04E-08	3.73E-07
Ovarian Cancer								
IGROV1	105	7	-42	-32	-28	3.63E-08	1.39E-07	>1.00E-04
OVCAR-3	109	-57	-51	-55	-61	2.27E-08	4.53E-08	9.05E-08
OVCAR-4	88	-88	-97	-99	-99	1.64E-08	3.15E-08	6.05E-08

Fig. 1d

	Log10 Concentration							
	Percent Growth							
Panel/Cell Line	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Ovarian Cancer								
OVCAR-5	101	-88	-95	100	-97	1.86E-08	3.42E-08	6.31E-08
OVCAR-8	101	-14	-85	-90	-67	2.79E-08	7.60E-08	3.22E-07
SK-OV-3	90	-1	-64	-48	.	2.75E-08	9.72E-08	
Renal Cancer								
786-0	96	-90	-96	-97	-90	1.77E-08	3.28E-08	6.09E-06
A498	98	34	13	-12	-45	5.66E-08	3.38E-06	>1.00E-04
ACHN	85	-68	-94	-95	-83	1.68E-08	3.57E-08	7.56E-08
CAKI-1								
RXF-393	84	-28	-54	-42	-22	2.02E-08	5.61E-08	
RXF-631								
SN12C	100	-91	-85	-92	-83	1.83E-08	3.34E-08	6.11E-08
TK-10	102	.	-94	-91	-86	3.38E-08	1.10E-07	3.58E-07
UO-31	114	-9	-41	-41	-23	3.30E-08	8.40E-08	>1.00E-04

Fig. 1e

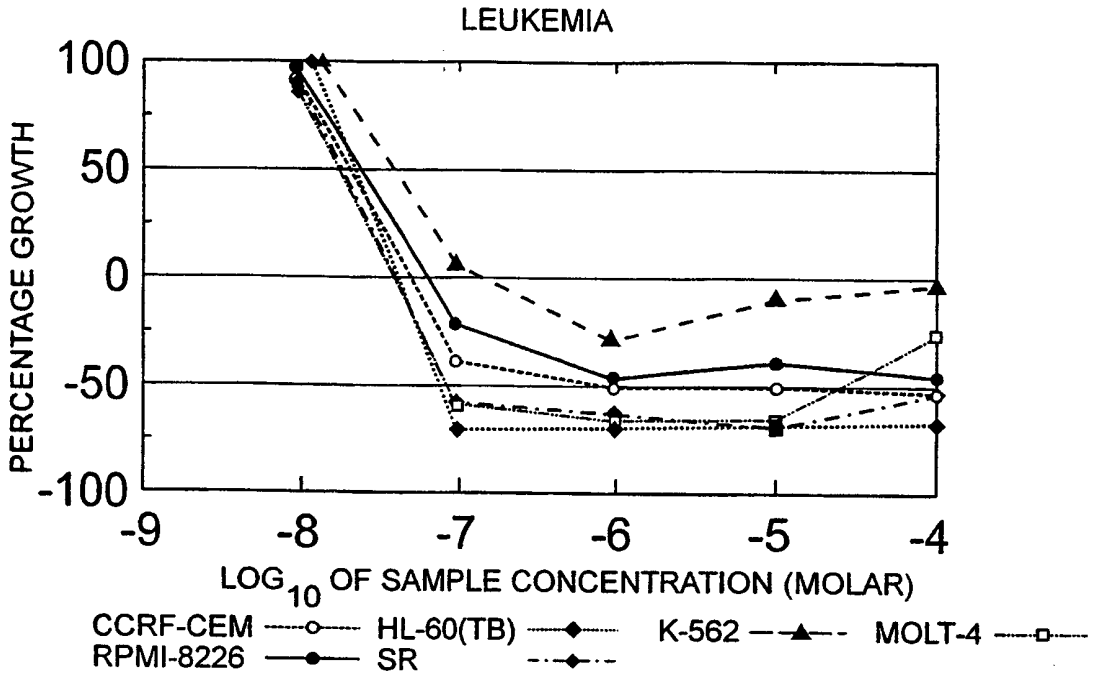

LEUKEMIA
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR

Fig. 1f

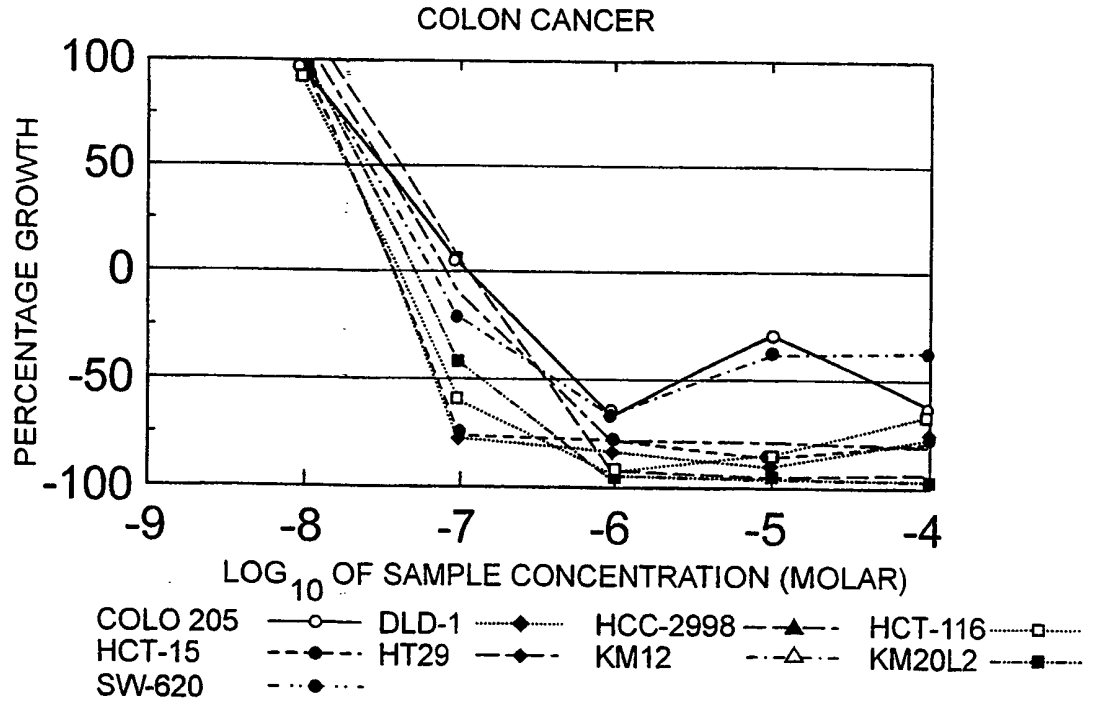

COLON CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
COLO 205
DLD-1
HCC-2998
HCT-116
HCT-15
HT29
KM12
KM20L2
SW-620

Fig. 1g

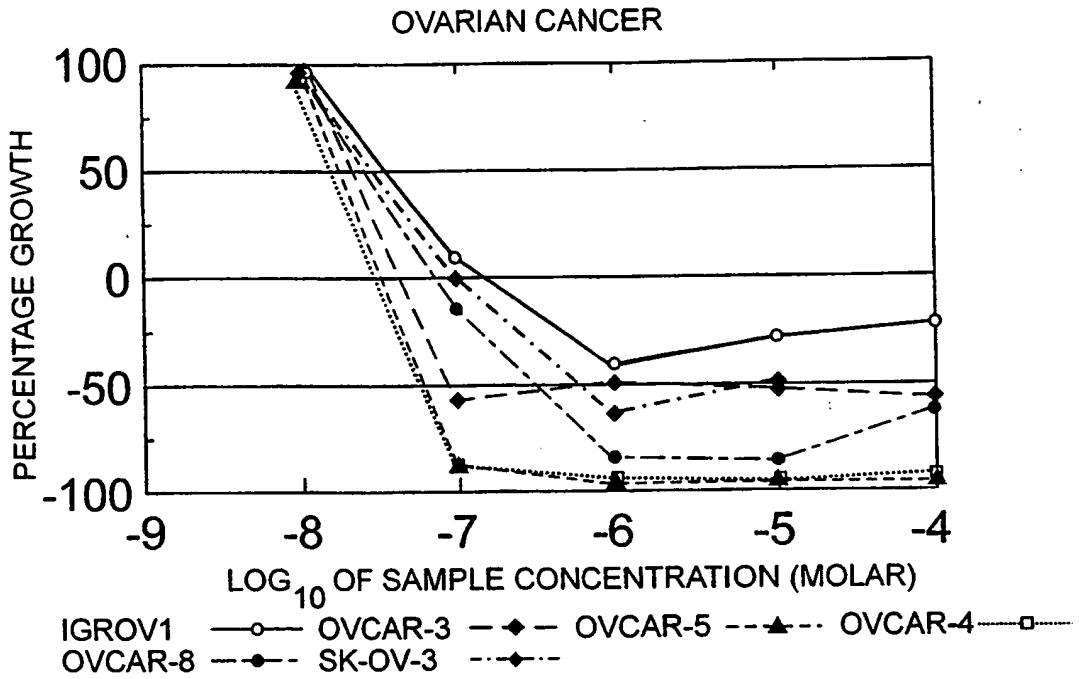

OVARIAN CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
IGROV1
OVCAR-3
OVCAR-5
OVCAR-4
OVCAR-8
SK-OV-3

Fig. 1h

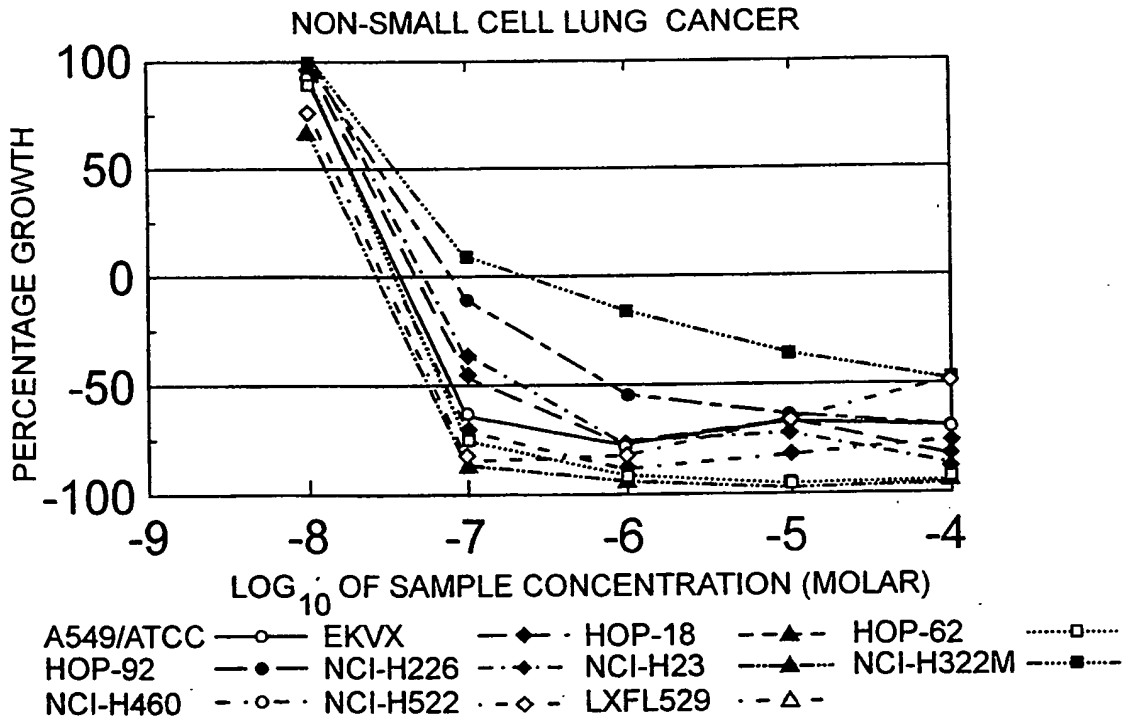

NON-SMALL CELL LUNG CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
A549/ATCC
EKVX
HOP-18
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522
LXFL529

Fig. 1i

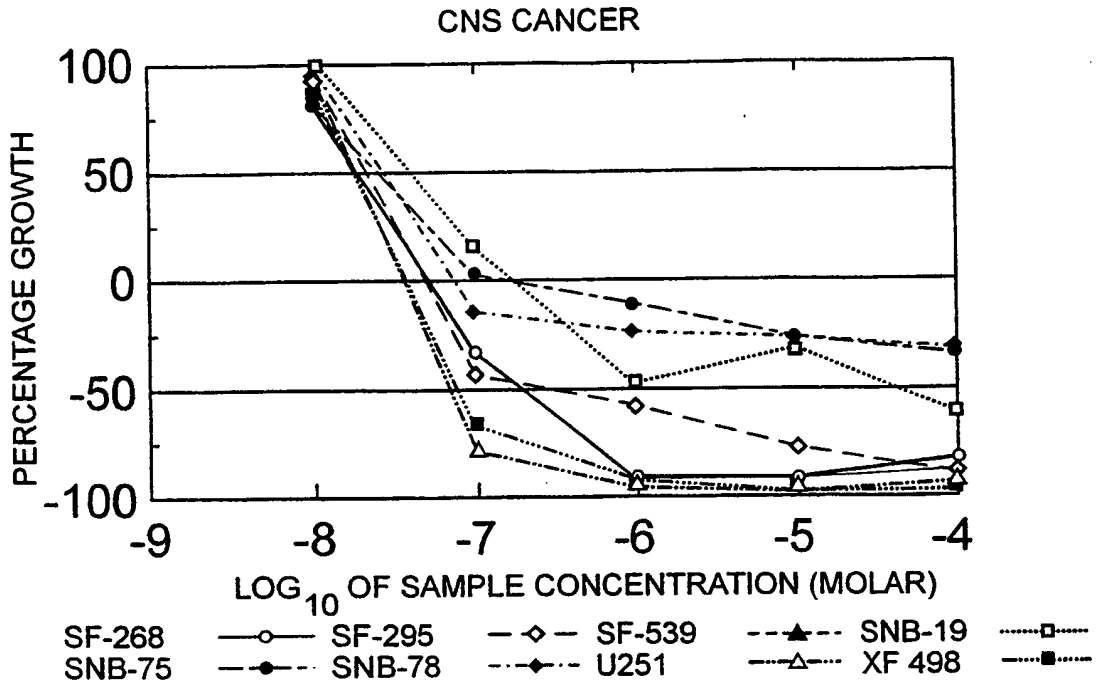

CNS CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
SF-268
SF-295
SF-539
SNB-19
SNB-75
SNB-78
U251
XF 498

Fig. 1j

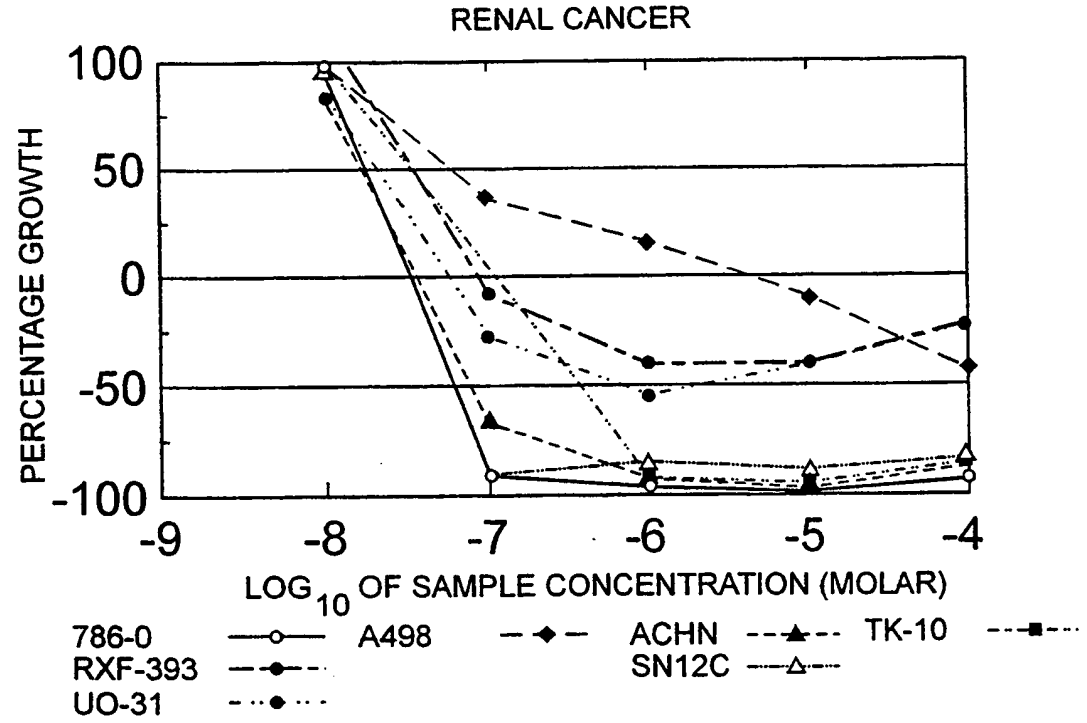

RENAL CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
786-0
A498
ACHN
TK-10
RXF-393
SN12C
UO-31

Fig. 1k

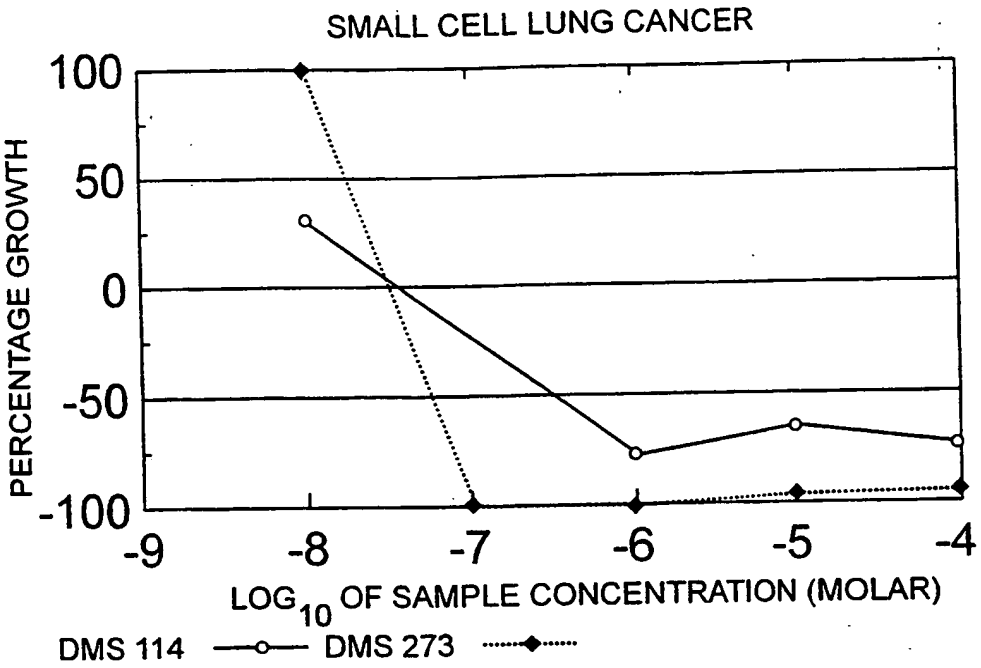

SMALL CELL LUNG CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
DMS 114
DMS 273

Fig. 1l

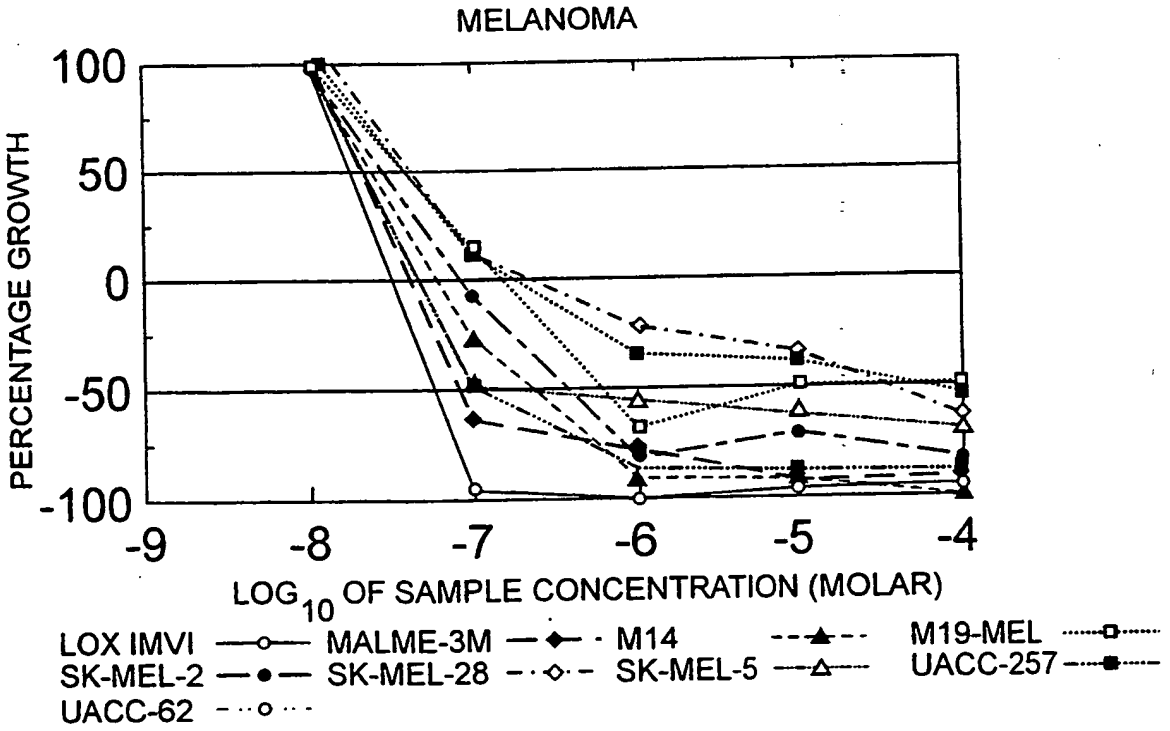

MELANOMA
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
LOX IMVI
MALME-3M
M14
M19-MEL
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62

Fig. 1m

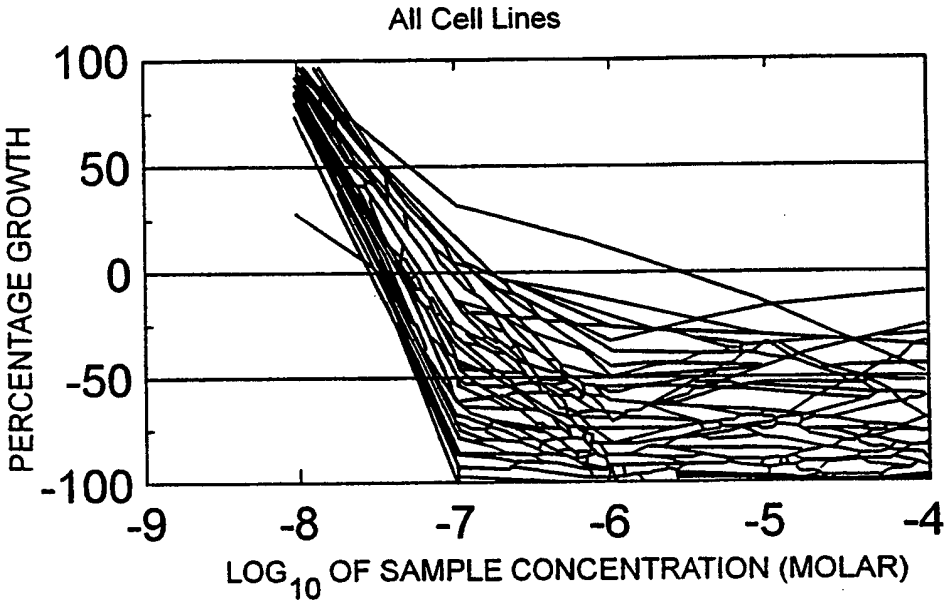

All Cell Lines
100
50
0
-50
-100
PERCENTAGE GROWTH
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)

Fig. 1n

National Cancer Institute Developmental Therapeutics Program	NSC: D-654033-O/I	Units: Molar SSPL:OCXW	Exp. ID: 9207SC8
Mean Graphs	Report Date: September 8, 1992	Test Date: July 20, 1992	
Panel/Cell Line	Log_{19} GI50 G150	Log_{19} TGI TGI	Log_{19} LC50 LC50
Leukemia			
CCRF-CEM	-7.70	-7.31	-6.17
HL-60(TB)	-7.67	-7.39	-7.12
K-562	-7.43	-6.87	> -4.00
MOLT-4	-7.74	-7.40	
RPMI-8226	-7.61	-7.20	> -4.00
SR	-7.76	-7.42	-7.07
Non-small Cell Lung Cancer			
A549/ATCC	-7.72	-7.41	-7.09
EKVX	-7.61	-7.30	-6.89
HOP-18			
HOP-62	-7.72	-7.43	-7.15
HOP-92	-7.54	-7.11	-6.17
NCI-H226	-7.59	-7.25	-6.70

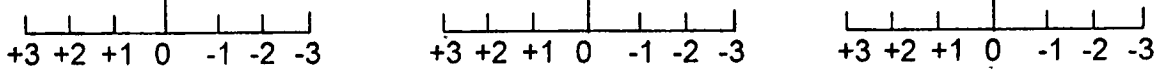
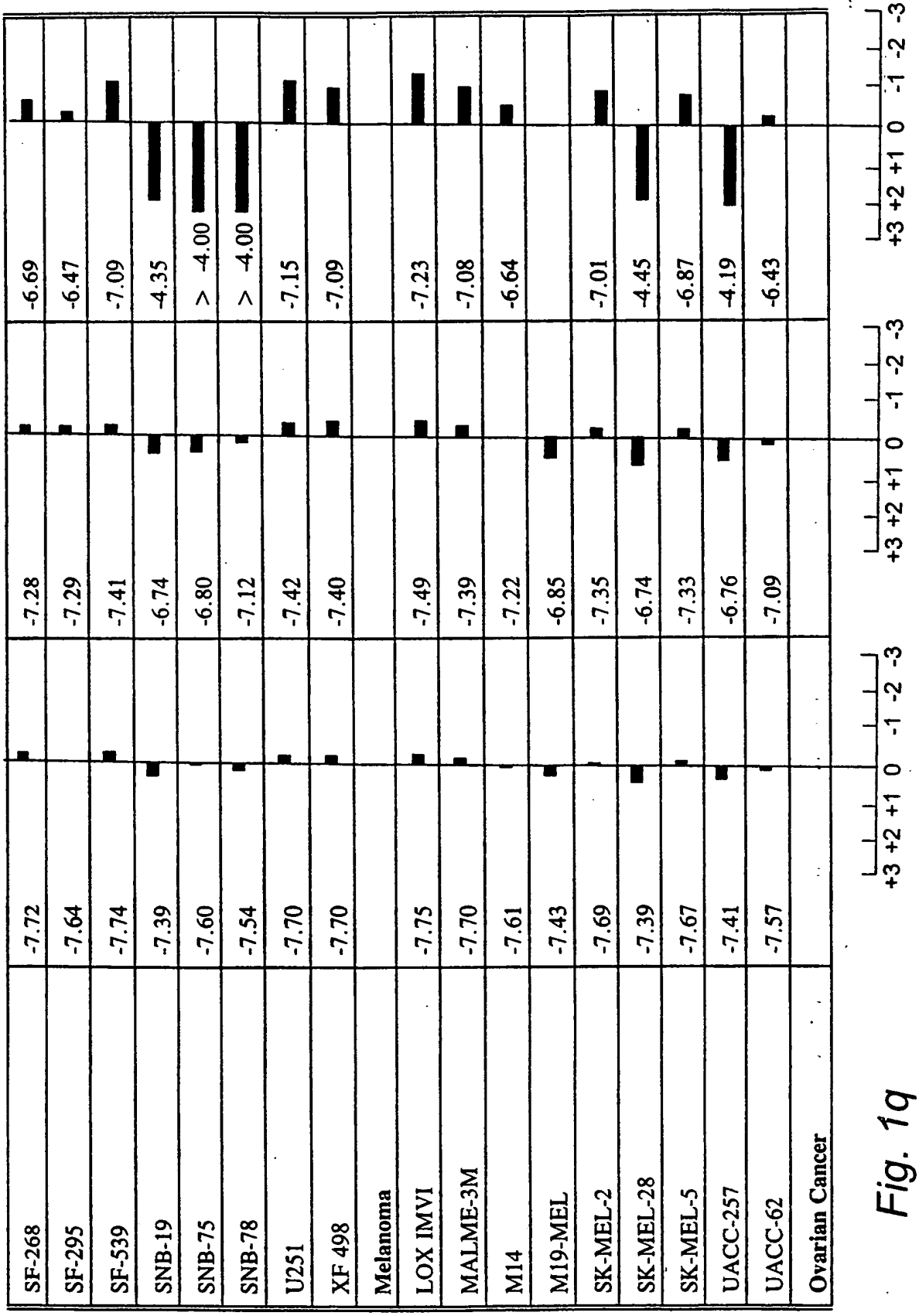


Fig. 1o

NCI-H23	-7.83	-7.52			-7.21	
NCI-H322M	-7.45	-6.75			-4.12	
NCI-H460	-7.74	-7.46			-7.19	
NCI-H522	-7.76	-7.45			-7.14	
LXFL 529	-7.73	-7.47			-7.20	
Small Cell Lung Cancer						
DMS 114	< -8.00	-7.43			-6.51	
DMS 273	-7.75	-7.50			-7.25	
Colon Cancer						
COLO 205	-7.53	-6.97			-7.16	
DLD-1	-7.73	-7.44			-6.43	
HCC-2998	-7.39	-6.94			-7.07	
HCT-116	-7.73	-7.40			-7.15	
HCT-15	-7.69	-7.42			-6.43	
HT29	-7.52	-7.09			-6.86	
KM12						
KM20L2	-7.64	-7.30				
SW-620	-7.60	-7.18				
CNS Cancer						

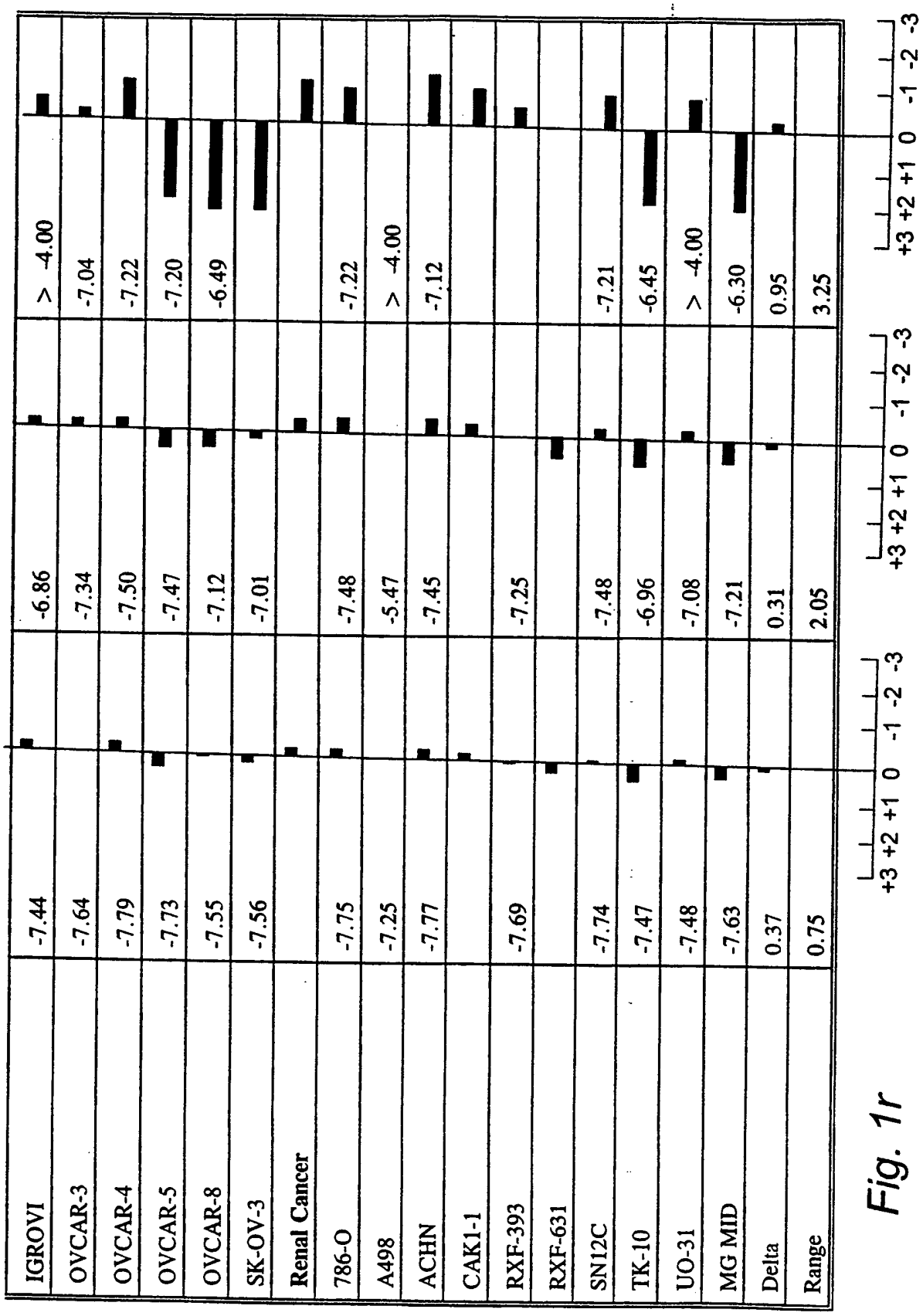
+3 +2 +1 0 -1 -2 -3

Fig. 1p



SF-268	-7.72	-7.28	-6.69	
SF-295	-7.64	-7.29	-6.47	
SF-539	-7.74	-7.41	-7.09	
SNB-19	-7.39	-6.74	-4.35	
SNB-75	-7.60	-6.80	> -4.00	
SNB-78	-7.54	-7.12	> -4.00	
U251	-7.70	-7.42	-7.15	
XF 498	-7.70	-7.40	-7.09	
Melanoma				
LOX IMVI	-7.75	-7.49	-7.23	
MALME-3M	-7.70	-7.39	-7.08	
M14	-7.61	-7.22	-6.64	
M19-MEL	-7.43	-6.85		
SK-MEL-2	-7.69	-7.35	-7.01	
SK-MEL-28	-7.39	-6.74	-4.45	
SK-MEL-5	-7.67	-7.33	-6.87	
UACC-257	-7.41	-6.76	-4.19	
UACC-62	-7.57	-7.09	-6.43	
Ovarian Cancer				

Fig. 1q



IGROVI	-7.44	-6.86	> -4.00	
OVCAR-3	-7.64	-7.34	-7.04	
OVCAR-4	-7.79	-7.50	-7.22	
OVCAR-5	-7.73	-7.47	-7.20	
OVCAR-8	-7.55	-7.12	-6.49	
SK-OV-3	-7.56	-7.01		
Renal Cancer				
786-O	-7.75	-7.48	-7.22	
A498	-7.25	-5.47	> -4.00	
ACHN	-7.77	-7.45	-7.12	
CAK1-1				
RXF-393	-7.69	-7.25		
RXF-631				
SN12C	-7.74	-7.48	-7.21	
TK-10	-7.47	-6.96	-6.45	
UO-31	-7.48	-7.08	> -4.00	
MG MID	-7.63	-7.21	-6.30	
Delta	0.37	0.31	0.95	
Range	0.75	2.05	3.25	

Fig. 1r

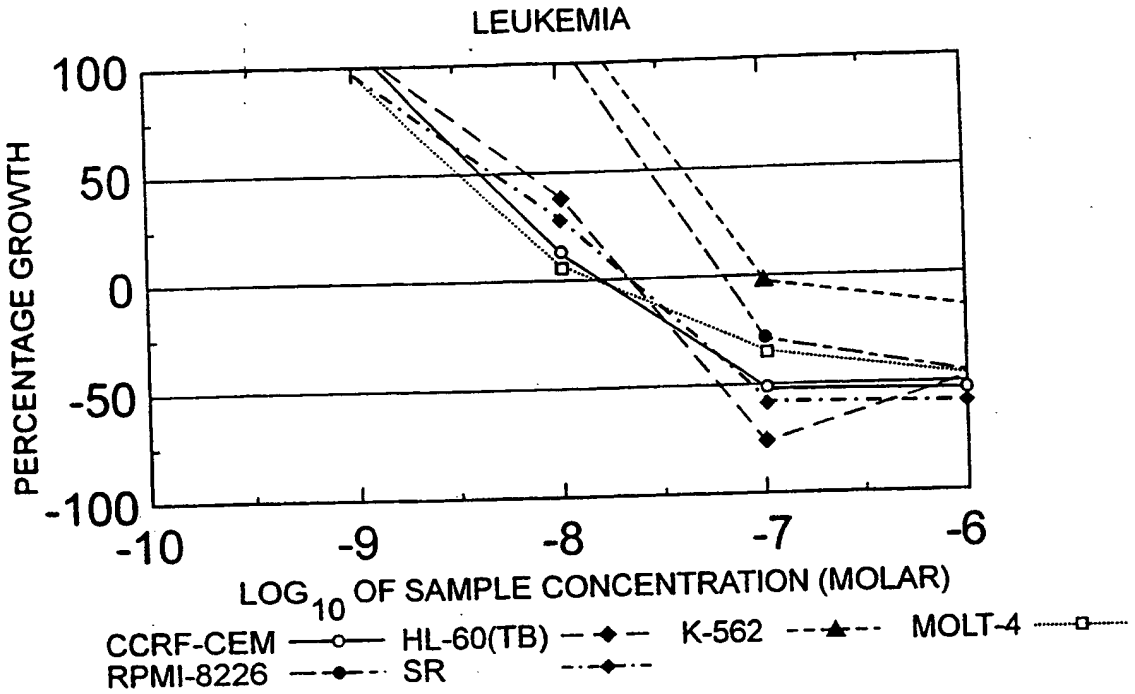

LEUKEMIA
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR

Fig. 2a

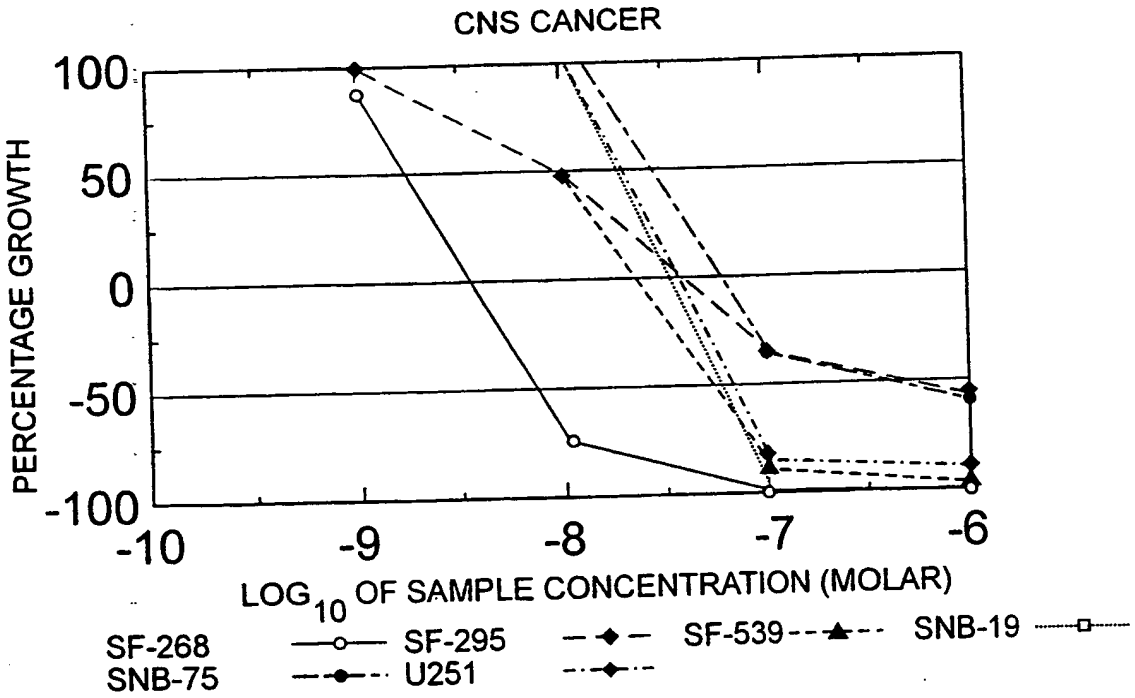

CNS CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251

Fig. 2b

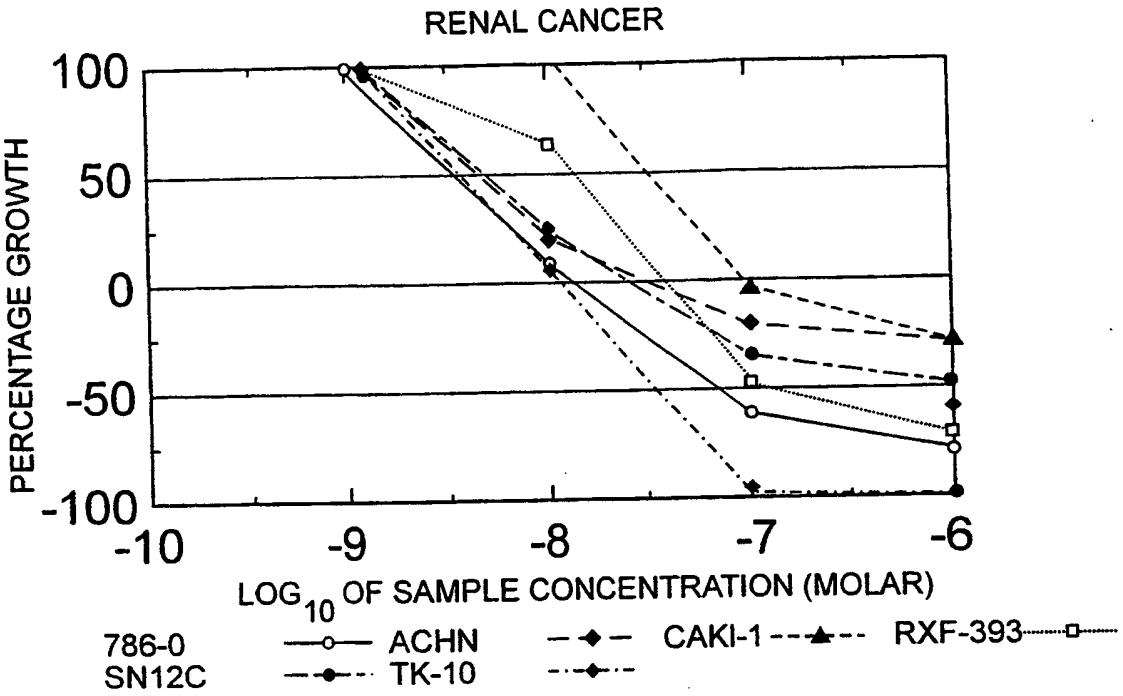

RENAL CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
786-0
ACHN
CAKI-1
RXF-393
SN12C
TK-10

Fig. 2c

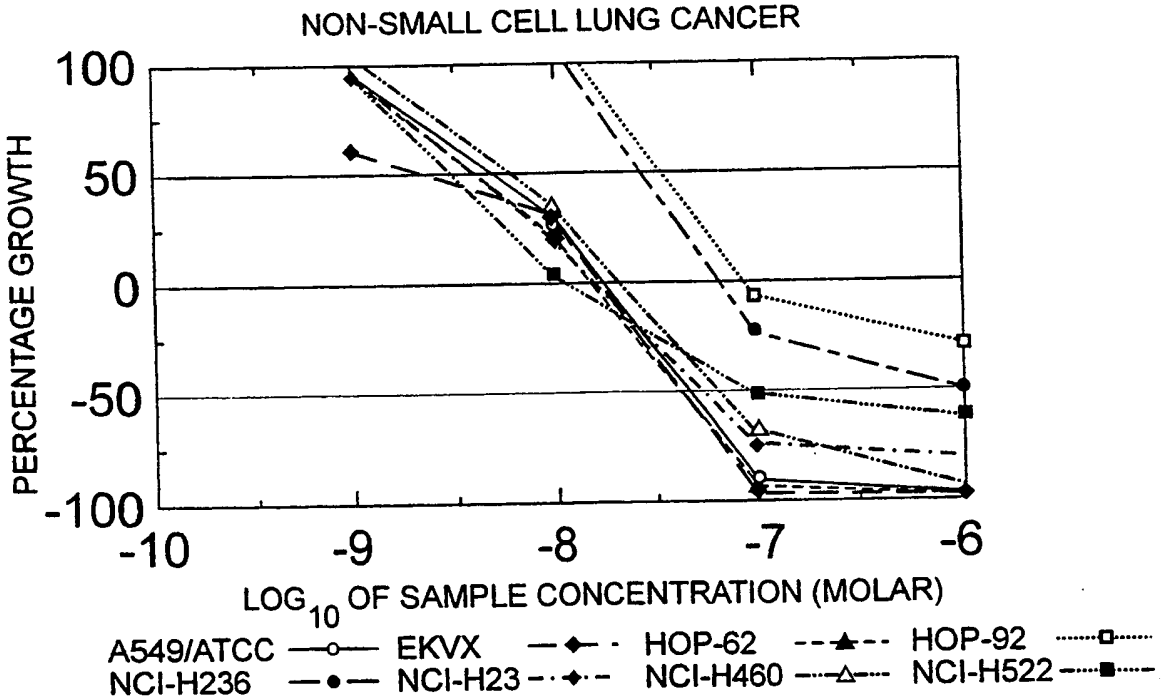

NON-SMALL CELL LUNG CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H236
NCI-H23
NCI-H460
NCI-H522

Fig. 2d

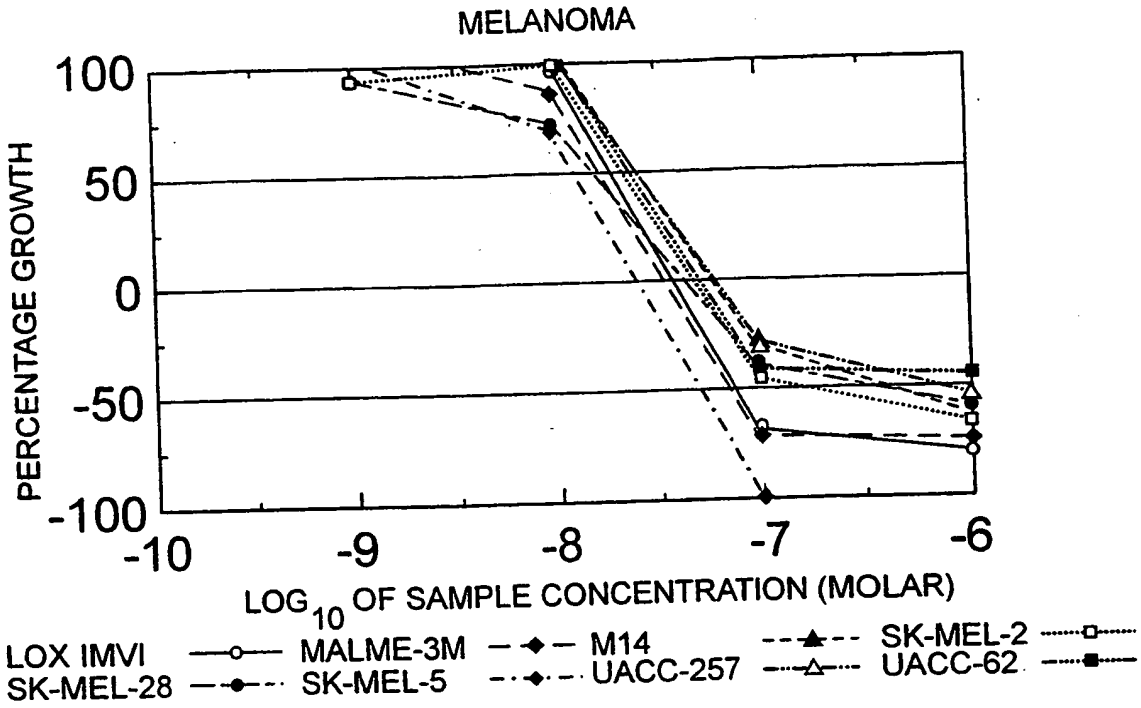

MELANOMA
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
LOX IMVI
MALME-3M
M14
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62

Fig. 2e

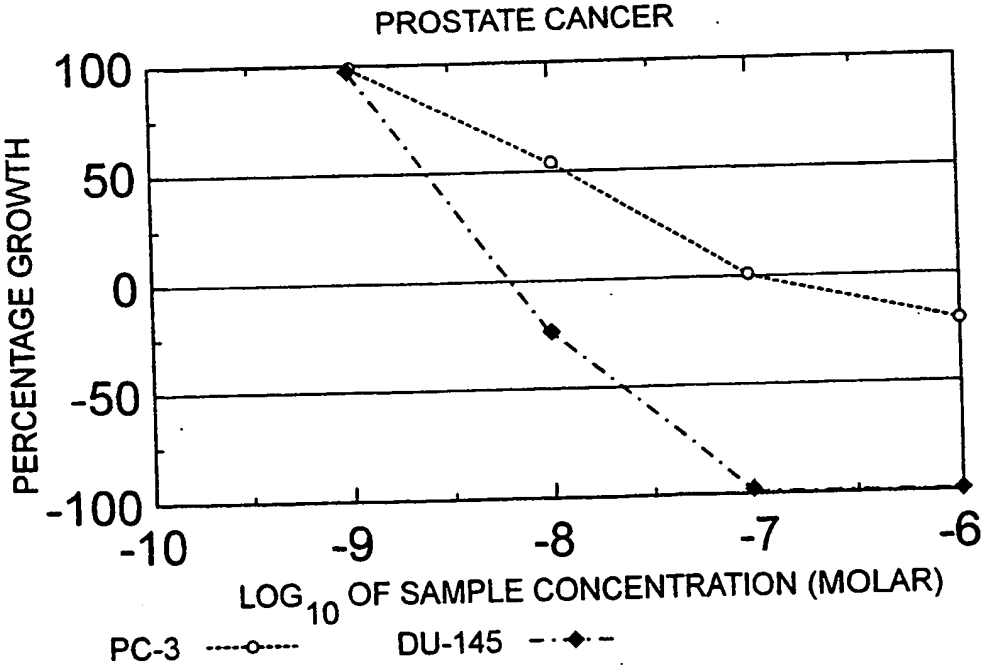

PROSTATE CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
PC-3
DU-145

Fig. 2f

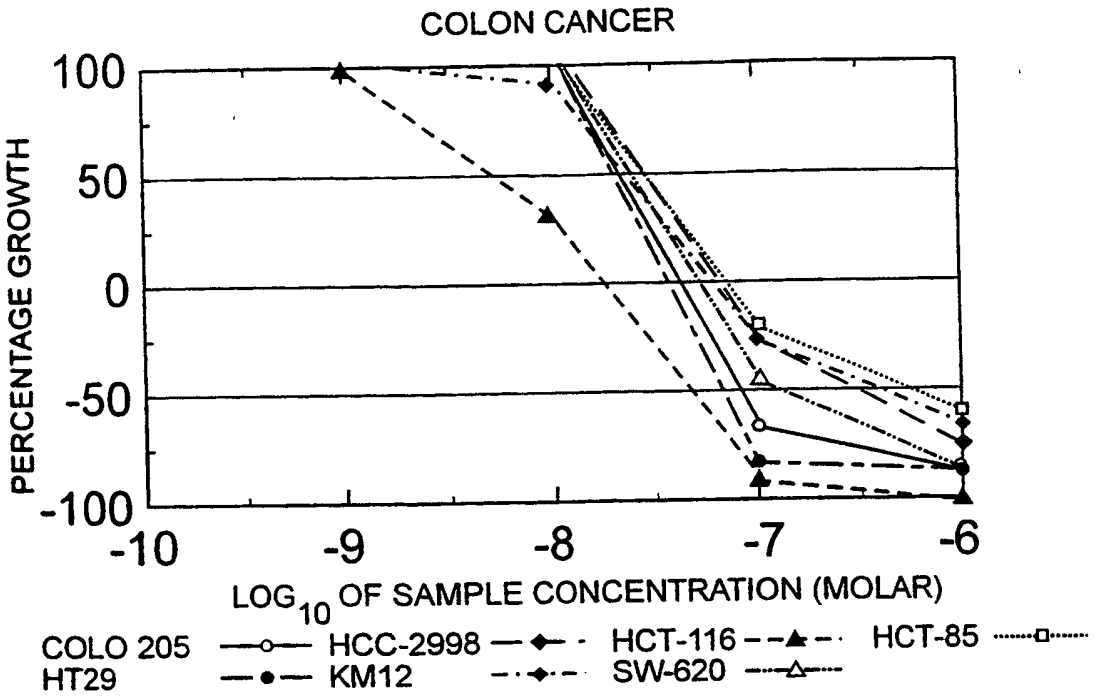

COLON CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
COLO 205
HCC-2998
HCT-116
HCT-85
HT29
KM12
SW-620

Fig. 2g

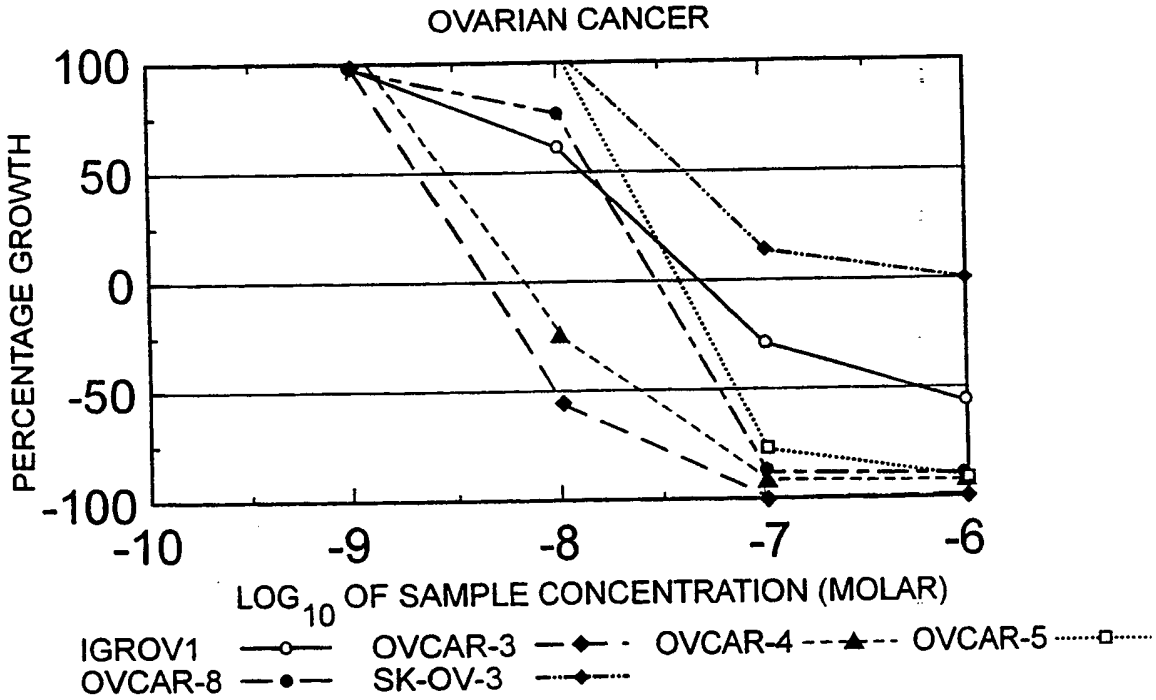

OVARIAN CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3

Fig. 2h

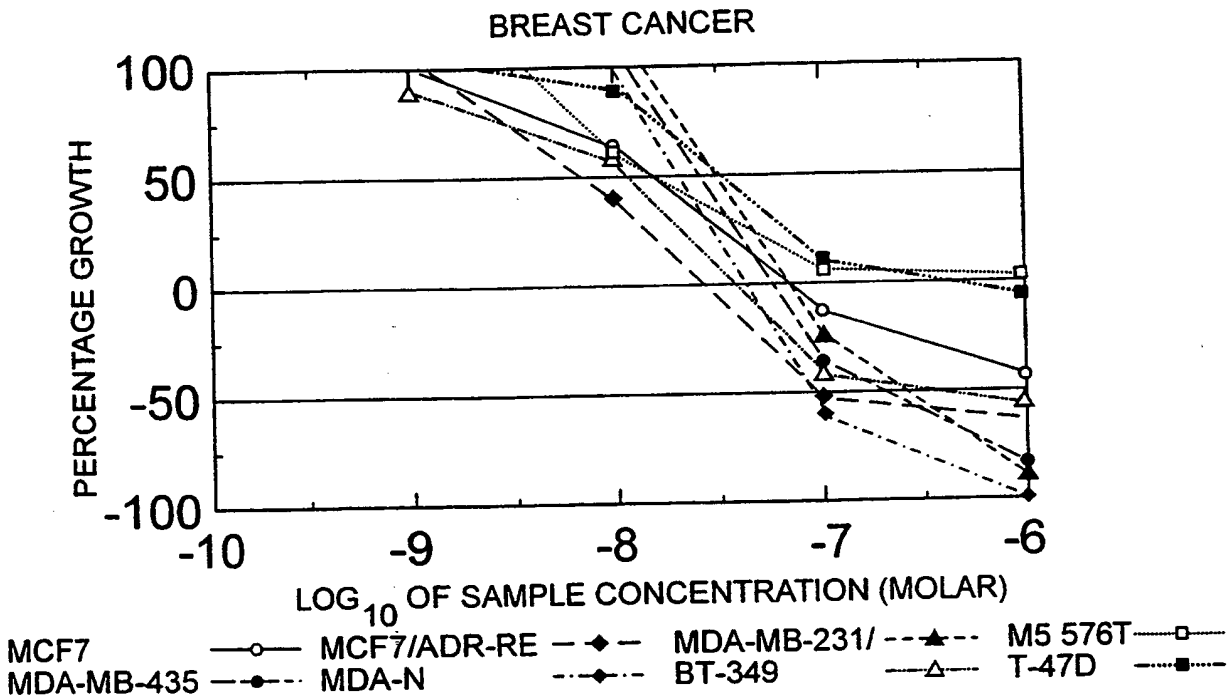

BREAST CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-10
-9
-8
-7
-6
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
MCF7
MCF7/ADR-RE
MDA-MB-231/
M5 576T
MDA-MB-435
MDA-N
BT-349
T-47D

Fig. 2i

National Cancer Institute Developmental Therapeutics Program	NSC: D-654033-O/0-2/2	Units: Molar SSPL:	Exp. ID: 9305MD56
Mean Graphs	Report Date: October 20, 1993	Test Date: May 18, 1993	
Panel/Cell Line	Log_{19} GI50 G150	Log_{19} TGI TGI	Log_{19} LC50 LC50
Leukemia			
CCRF-CEM	-8.42	-7.88	-7.06
HL-60(TB)	-8.18	-7.68	-7.25
K-562	-7.42	-7.03	
MOLT-4	-8.51	-7.90	-6.04
RPMI-8226	-7.59	-7.24	-6.03
SR	-8.34	-7.69	-7.12
Non-small Cell Lung Cancer			
A549/ATCC	-8.29	-7.73	-7.31
EKVX	-8.63	-7.75	-7.36
HOP-62	-8.32	-7.79	-7.35
HOP-92	-7.45	-7.04	
NCI-H226	-7.54	-7.16	

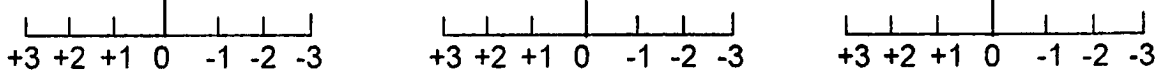


Fig. 2j

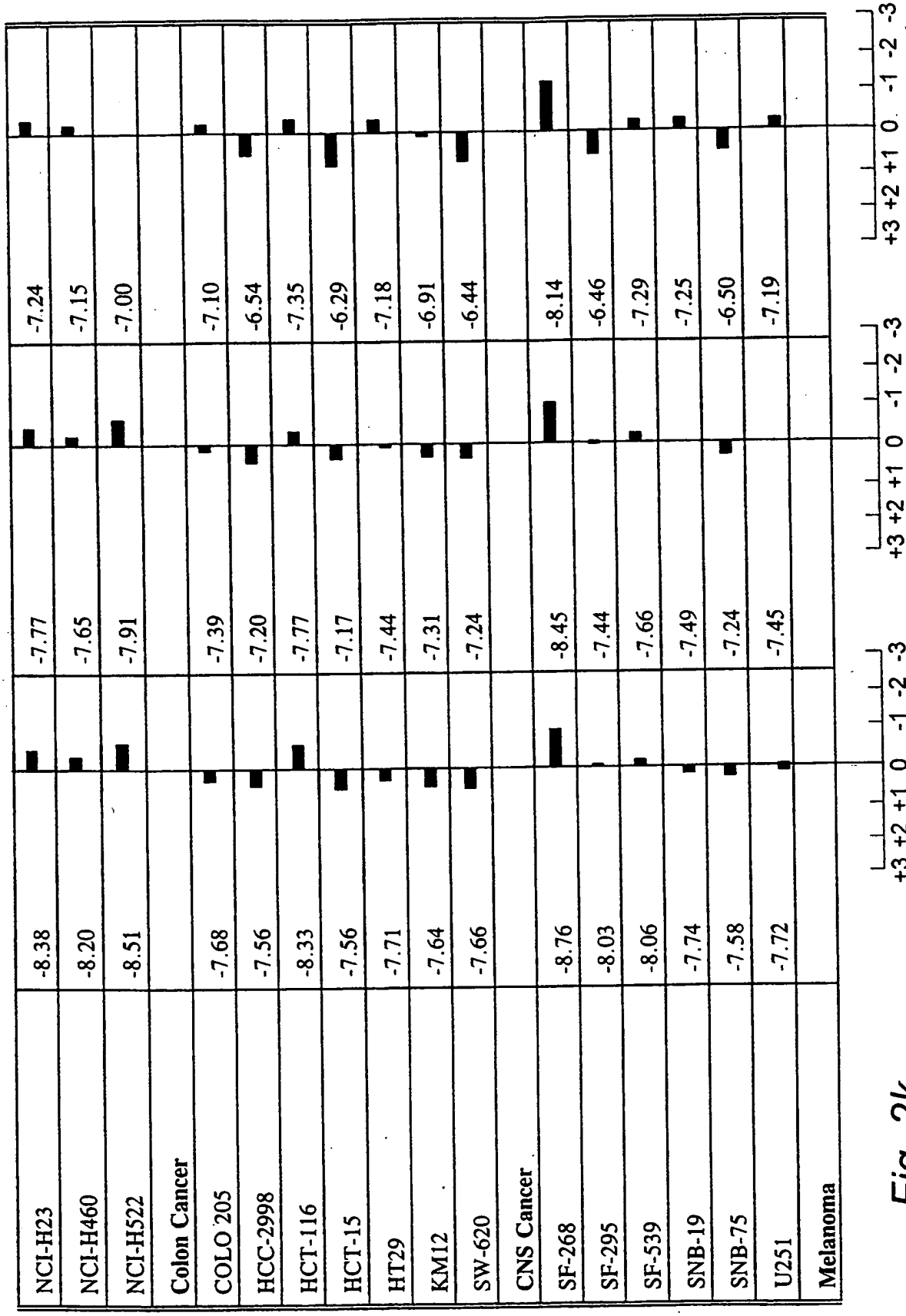

NCI-H23 -8.38 -7.77 -7.24
NCI-H460 -8.20 -7.65 -7.15
NCI-H522 -8.51 -7.91 -7.00
Colon Cancer
COLO 205 -7.68 -7.39 -7.10
HCC-2998 -7.56 -7.20 -6.54
HCT-116 -8.33 -7.77 -7.35
HCT-15 -7.56 -7.17 -6.29
HT29 -7.71 -7.44 -7.18
KM12 -7.64 -7.31 -6.91
SW-620 -7.66 -7.24 -6.44
CNS Cancer
SF-268 -8.76 -8.45 -8.14
SF-295 -8.03 -7.44 -6.46
SF-539 -8.06 -7.66 -7.29
SNB-19 -7.74 -7.49 -7.25
SNB-75 -7.58 -7.24 -6.50
U251 -7.72 -7.45 -7.19
Melanoma
+3 +2 +1 0 -1 -2 -3
+3 +2 +1 0 -1 -2 -3
+3 +2 +1 0 -1 -2 -3

Fig. 2k

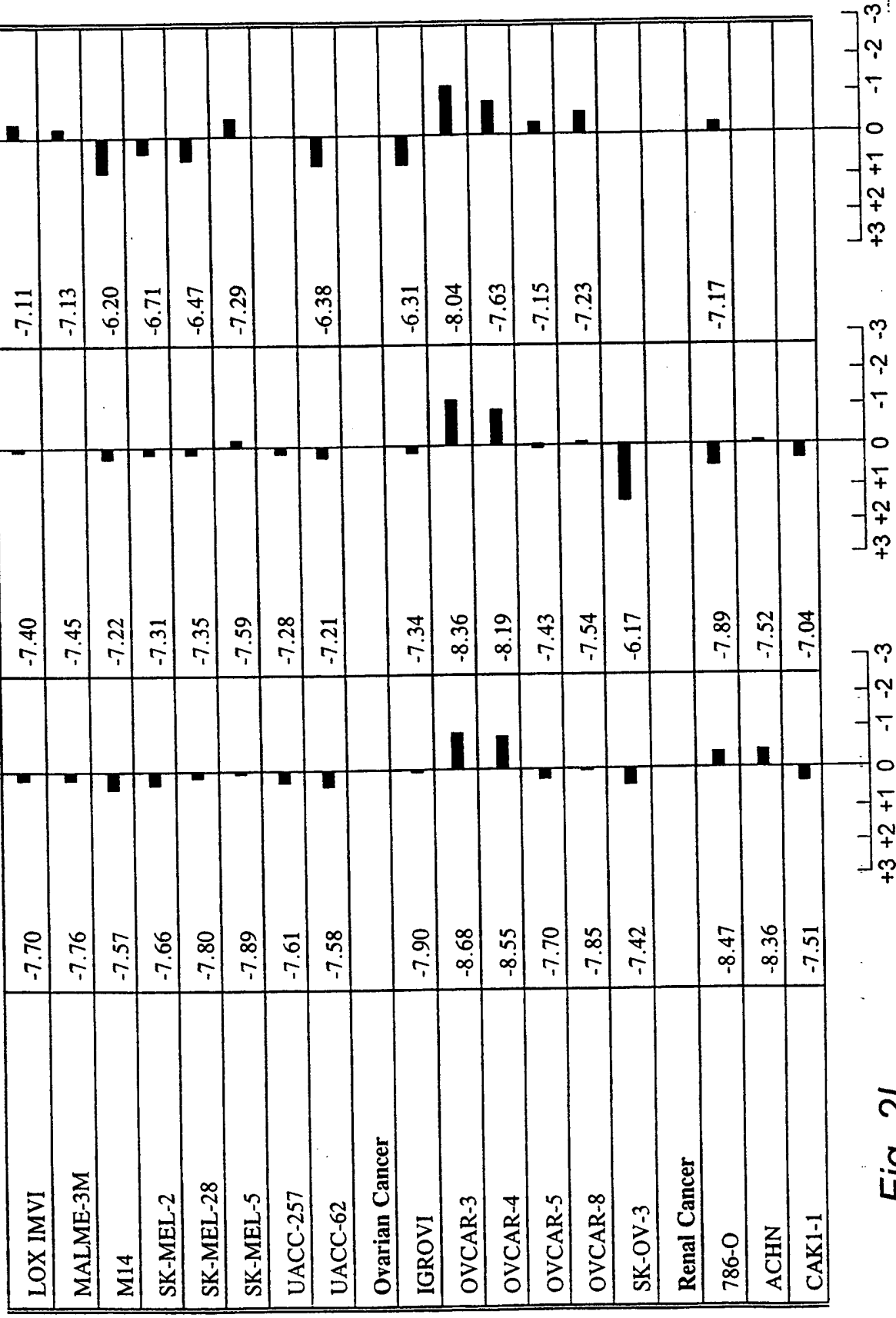
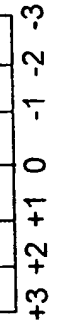
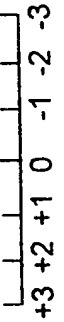

LOX IMVI -7.70 -7.40 -7.11
MALME-3M -7.76 -7.45 -7.13
M14 -7.57 -7.22 -6.20
SK-MEL-2 -7.66 -7.31 -6.71
SK-MEL-28 -7.80 -7.35 -6.47
SK-MEL-5 -7.89 -7.59 -7.29
UACC-257 -7.61 -7.28
UACC-62 -7.58 -7.21 -6.38
Ovarian Cancer
IGROVI -7.90 -7.34 -6.31
OVCAR-3 -8.68 -8.36 -8.04
OVCAR-4 -8.55 -8.19 -7.63
OVCAR-5 -7.70 -7.43 -7.15
OVCAR-8 -7.85 -7.54 -7.23
SK-OV-3 -7.42 -6.17
Renal Cancer
786-O -8.47 -7.89 -7.17
ACHN -8.36 -7.52
CAK1-1 -7.51 -7.04
+3 +2 +1 0 -1 -2 -3

Fig. 2I

RXF-393	-7.88	-7.43		-6.91	
SN12C	-8.32	-7.60			
TK-10	-8.44	-7.96		-7.46	
Prostate Cancer					
PC-3	-7.94	-6.99			
DU-145	-8.62	-8.19		-7.65	
Breast Cancer					
MCF7	-7.82	-7.15			
MCF7/ADR-RES	-8.15	-7.55		-6.92	
MDA-MB-231/ATCC	-7.52	-7.16		-6.57	
HS 578T	-7.81				
MDA-MB-435	-7.59	-7.24		-6.67	
MDA-N	-7.69	-7.37		-7.05	
BT-549	-7.92	-7.40		-6.24	
T-47D	-7.51	-6.36			
MG MID	-7.95	-7.47		-6.97	
Delta	0.81	0.98		1.17	
Range	1.34	2.28		2.11	





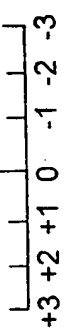


Fig. 2m

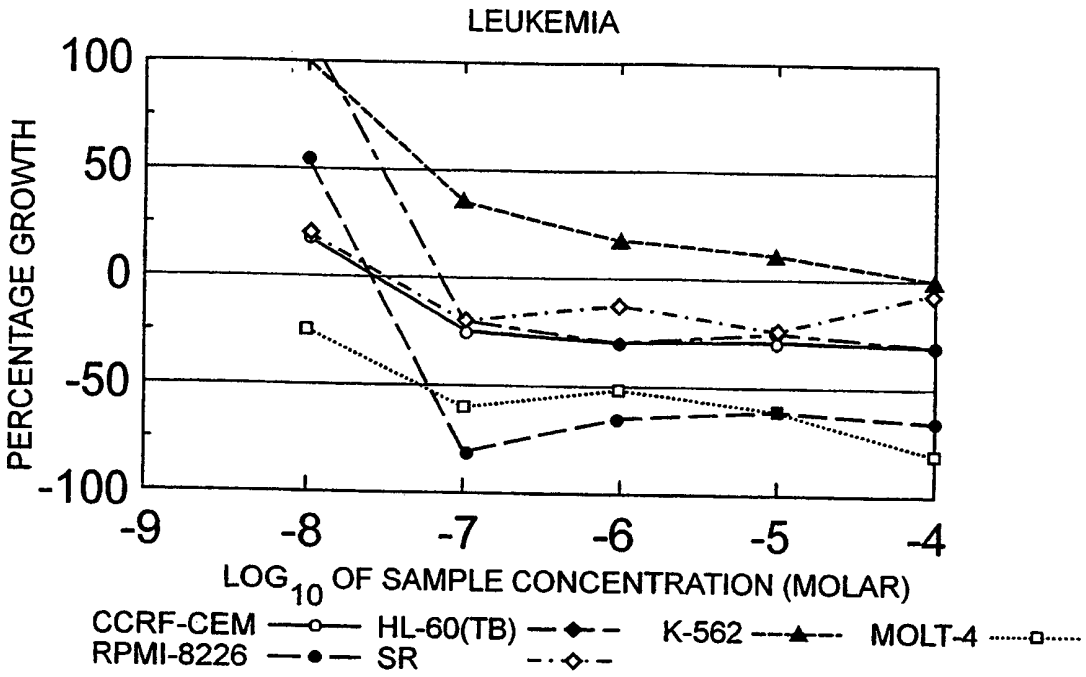

LEUKEMIA
100
50
0
-50
-100
PERCENTAGE GROWTH
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR

Fig. 3a

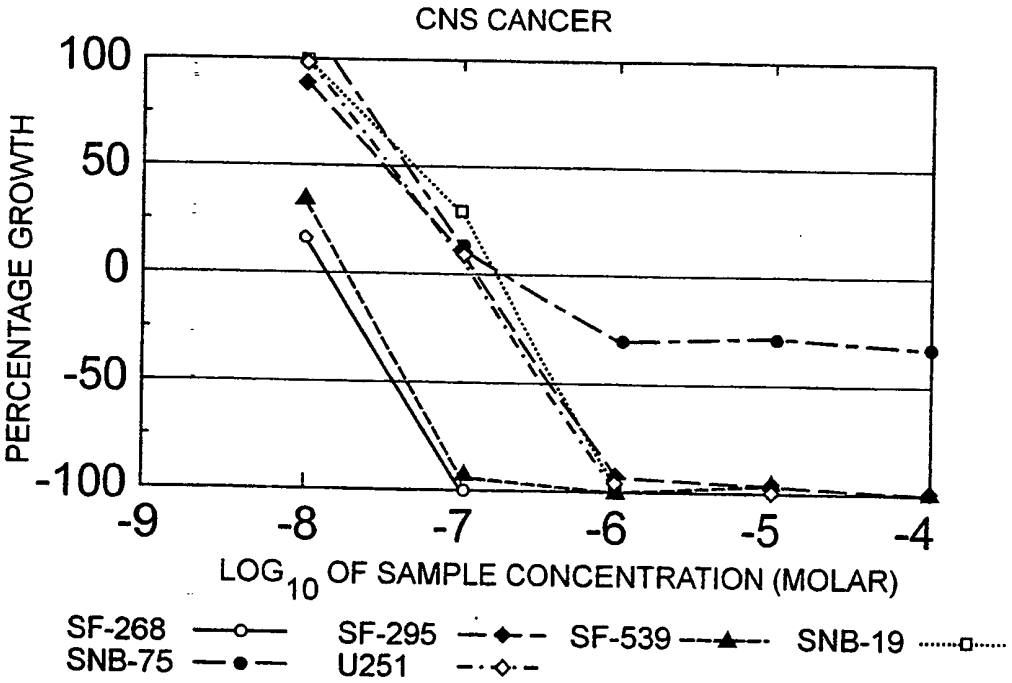

CNS CANCER
100
50
0
-50
-100
PERCENTAGE GROWTH
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251

Fig. 3b

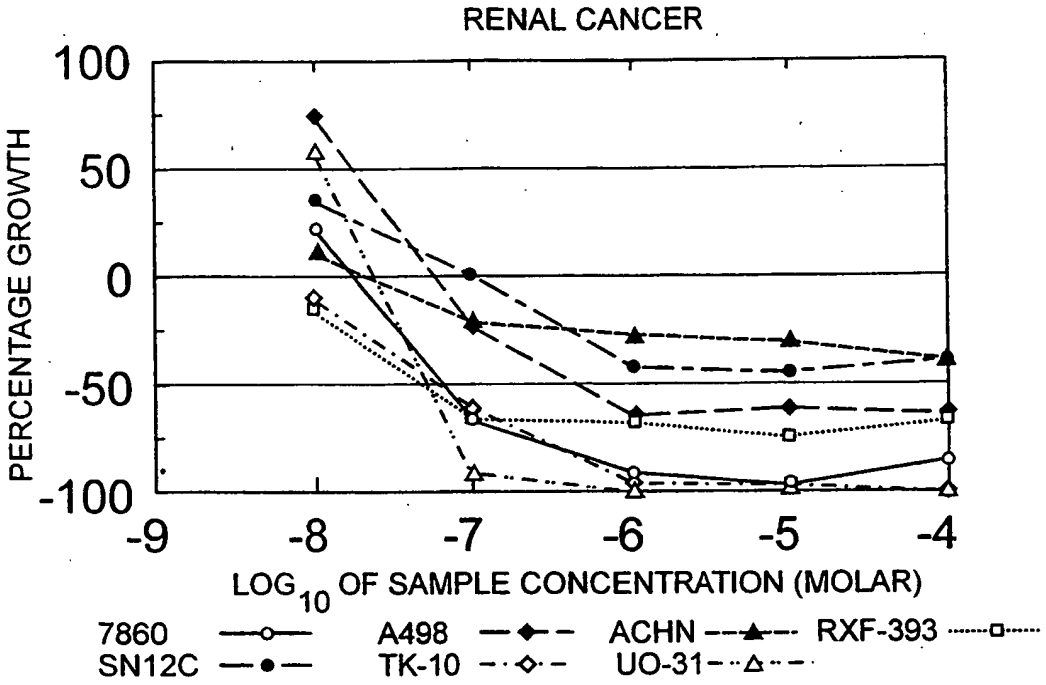

RENAL CANCER
100
50
0
-50
-100
PERCENTAGE GROWTH
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
7860
A498
ACHN
RXF-393
SN12C
TK-10
UO-31

Fig. 3c

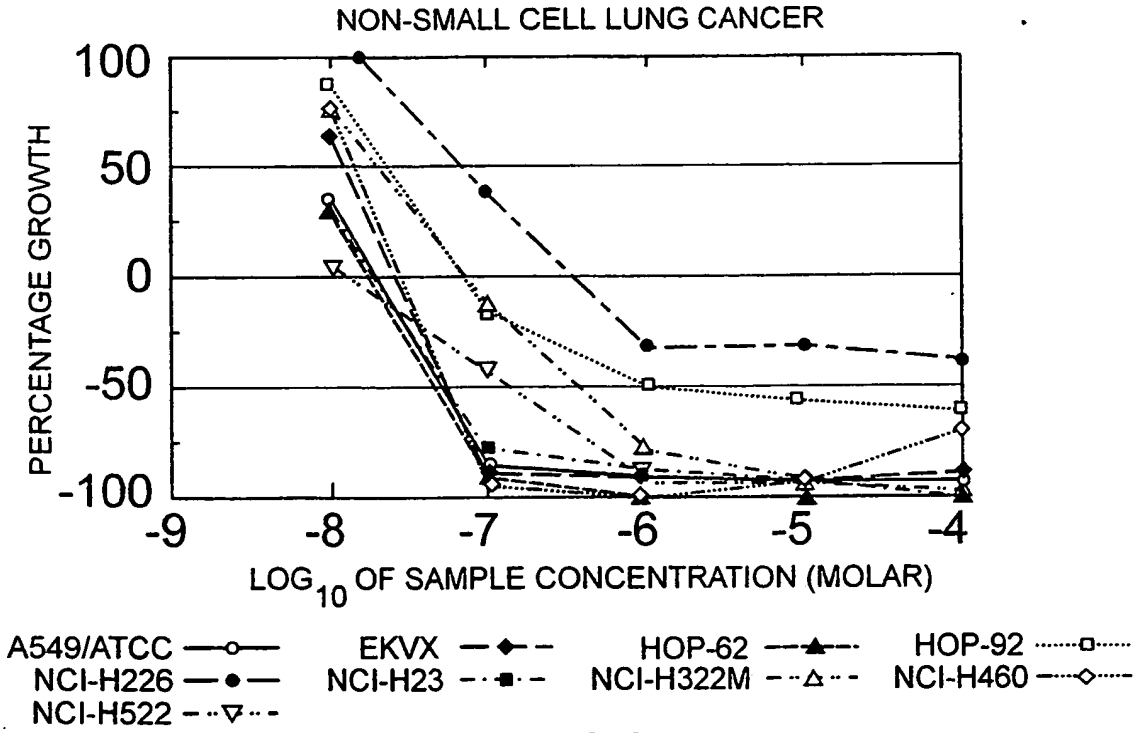

NON-SMALL CELL LUNG CANCER
100
50
0
-50
-100
PERCENTAGE GROWTH
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522

Fig. 3d

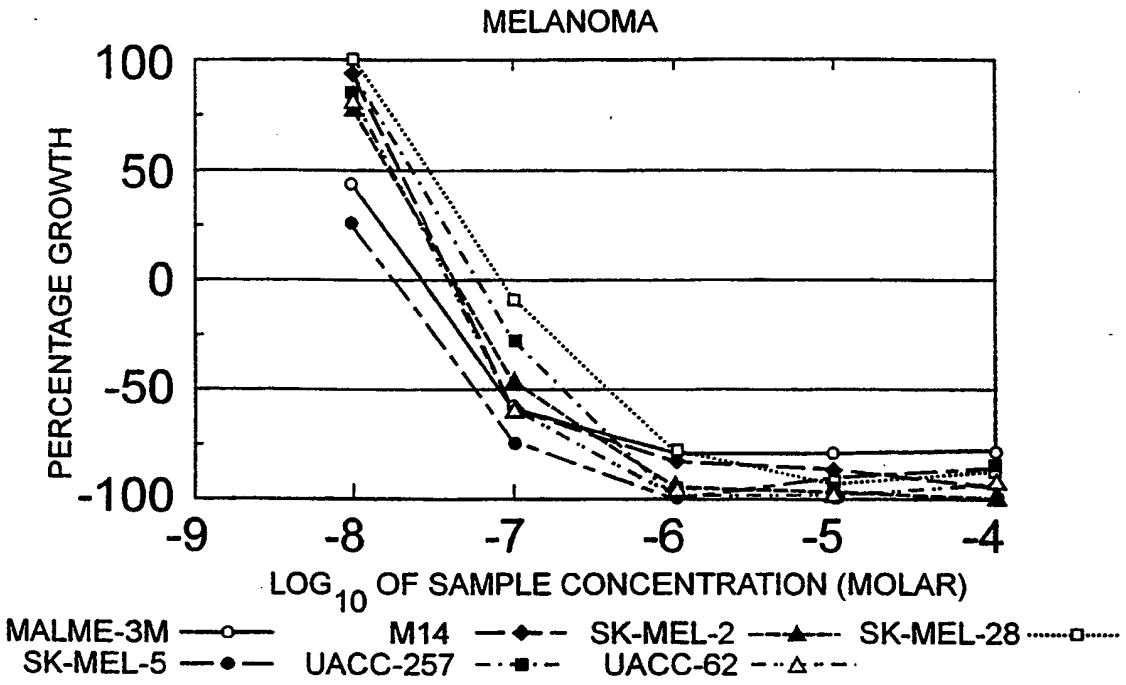

MELANOMA
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
MALME-3M
M14
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62

Fig. 3e

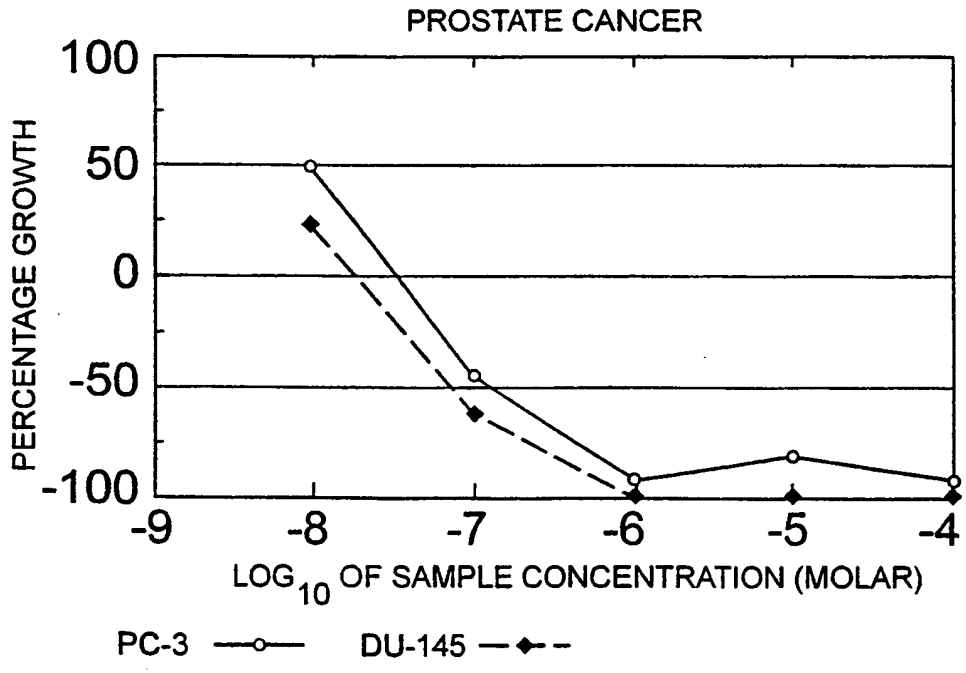

PROSTATE CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
PC-3
DU-145

Fig. 3f

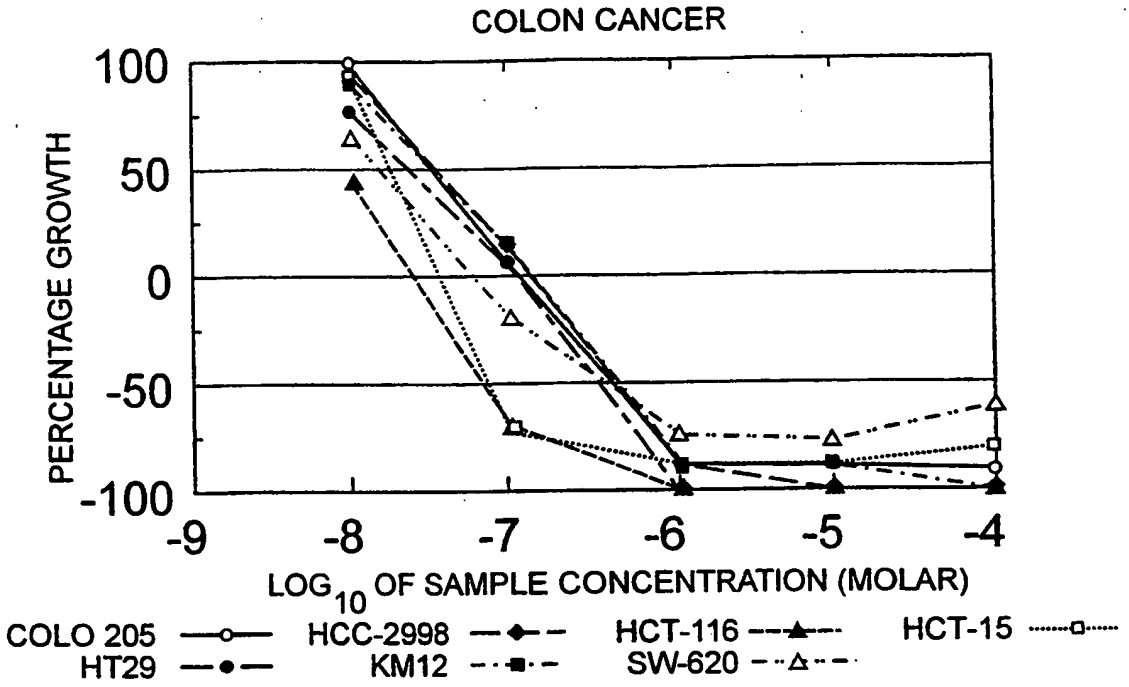

COLON CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
COLO 205
HCC-2998
HCT-116
HCT-15
HT29
KM12
SW-620

Fig. 3g

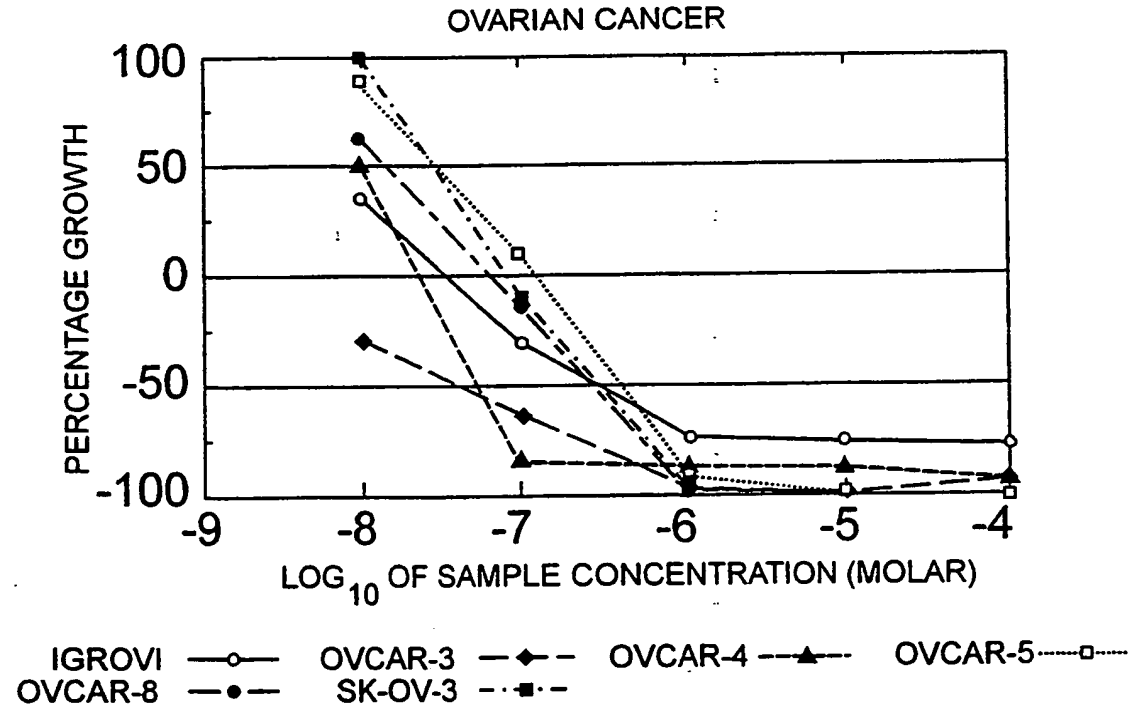

OVARIAN CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
IGROVI
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3

Fig. 3h

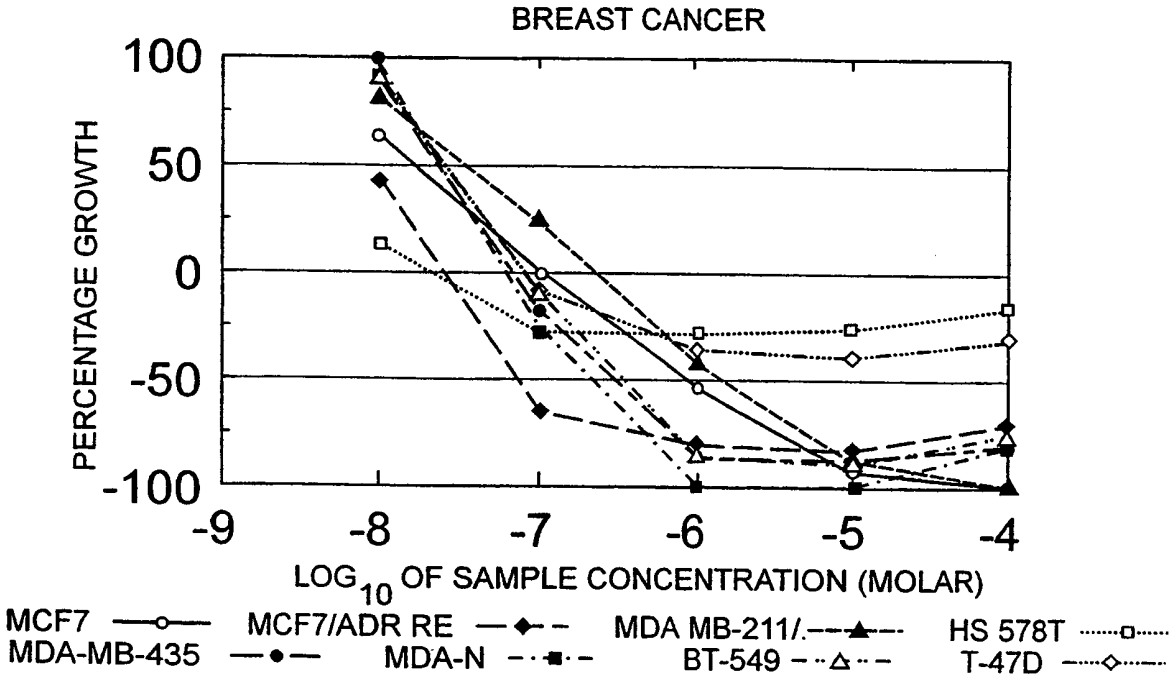

BREAST CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
MCF7
MCF7/ADR RE
MDA MB-211/.
HS 578T
MDA-MB-435
MDA-N
BT-549
T-47D

Fig. 3i

National Cancer Institute Developmental Therapeutics Program	NSC: D-659471-Y/0-1/5	Units: Molar SSPL:	Exp. ID: 9302MD33
Mean Graphs - Uscharidin	Report Date: March 8, 1993	Test Date: February 23, 1993	
Panel/Cell Line	Log_{10} GI50 G150	Log_{10} TGI TGI	Log_{10} LC50 LC50
Leukemia			
CCRF-CEM	< -8.00	-7.64	> -4.00
HL-60(TB)	-7.97	-7.59	-7.22
K-562	-7.25	> -4.00	> -4.00
MOLT-4	< -8.00	< -8.00	-7.35
RPMI-8226	-7.51	-7.15	> -4.00
SR	< -8.00	-7.55	> -4.00
Non-small Cell Lung Cancer			
A549/ATCC	< -8.00	-7.71	-7.29
EKVX	-7.91	-7.58	-7.25
HOP-62	< -8.00	-7.74	7.32
HOP-92	-7.65	-7.13	5.77
NCI-H226	-7.15	-6.44	> -4.00
NCI-H23	< -8.00	-7.74	-7.27

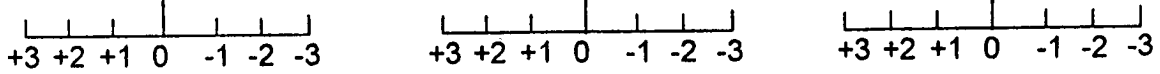


Fig. 3j

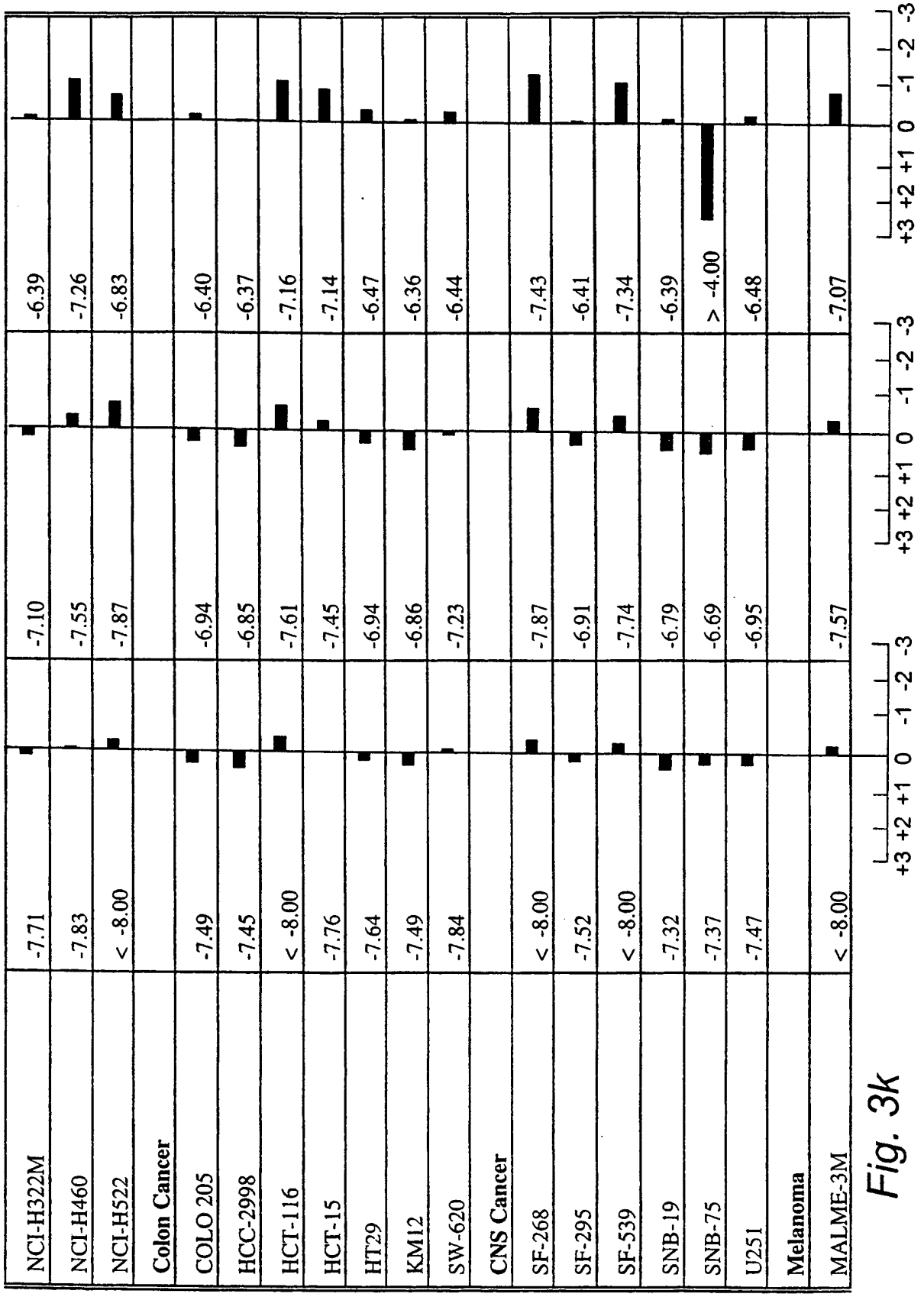

NCI-H322M -7.71 -7.10 -6.39
NCI-H460 -7.83 -7.55 -7.26
NCI-H522 < -8.00 -7.87 -6.83
Colon Cancer
COLO 205 -7.49 -6.94 -6.40
HCC-2998 -7.45 -6.85 -6.37
HCT-116 < -8.00 -7.61 -7.16
HCT-15 -7.76 -7.45 -7.14
HT29 -7.64 -6.94 -6.47
KM12 -7.49 -6.86 -6.36
SW-620 -7.84 -7.23 -6.44
CNS Cancer
SF-268 < -8.00 -7.87 -7.43
SF-295 -7.52 -6.91 -6.41
SF-539 < -8.00 -7.74 -7.34
SNB-19 -7.32 -6.79 -6.39
SNB-75 -7.37 -6.69 > -4.00
U251 -7.47 -6.95 -6.48
Melanoma
MALME-3M < -8.00 -7.57 -7.07
+3 +2 +1 0 -1 -2 -3

Fig. 3k

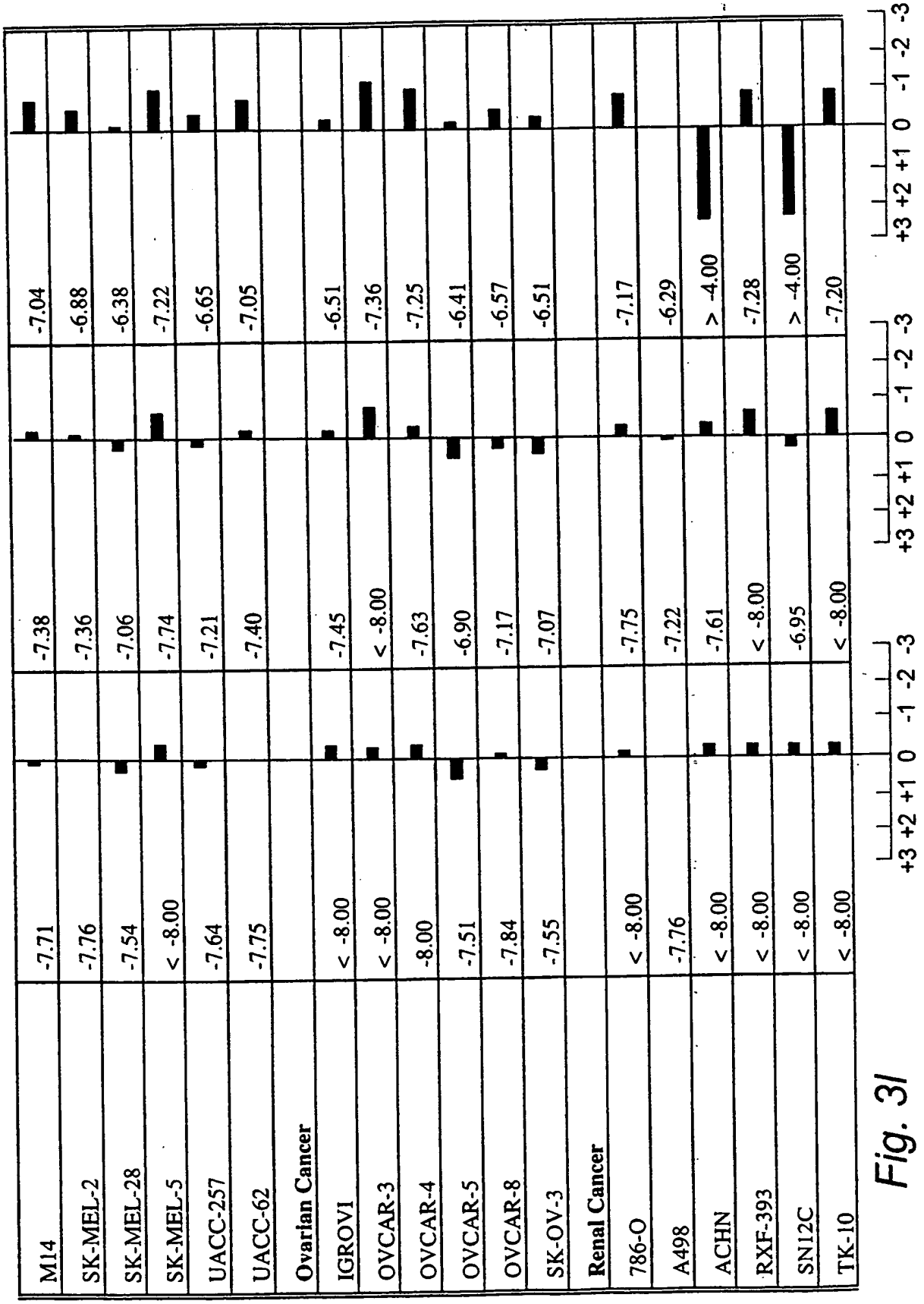

M14 -7.71 -7.38 -7.04
SK-MEL-2 -7.76 -7.36 -6.88
SK-MEL-28 -7.54 -7.06 -6.38
SK-MEL-5 < -8.00 -7.74 -7.22
UACC-257 -7.64 -7.21 -6.65
UACC-62 -7.75 -7.40 -7.05
Ovarian Cancer
IGROV1 < -8.00 -7.45 -6.51
OVCAR-3 < -8.00 < -8.00 -7.36
OVCAR-4 -8.00 -7.63 -7.25
OVCAR-5 -7.51 -6.90 -6.41
OVCAR-8 -7.84 -7.17 -6.57
SK-OV-3 -7.55 -7.07 -6.51
Renal Cancer
786-O < -8.00 -7.75 -7.17
A498 -7.76 -7.22 -6.29
ACHN < -8.00 -7.61 > -4.00
RXF-393 < -8.00 < -8.00 -7.28
SN12C < -8.00 -6.95 > -4.00
TK-10 < -8.00 < -8.00 -7.20
+3 +2 +1 0 -1 -2 -3

Fig. 31

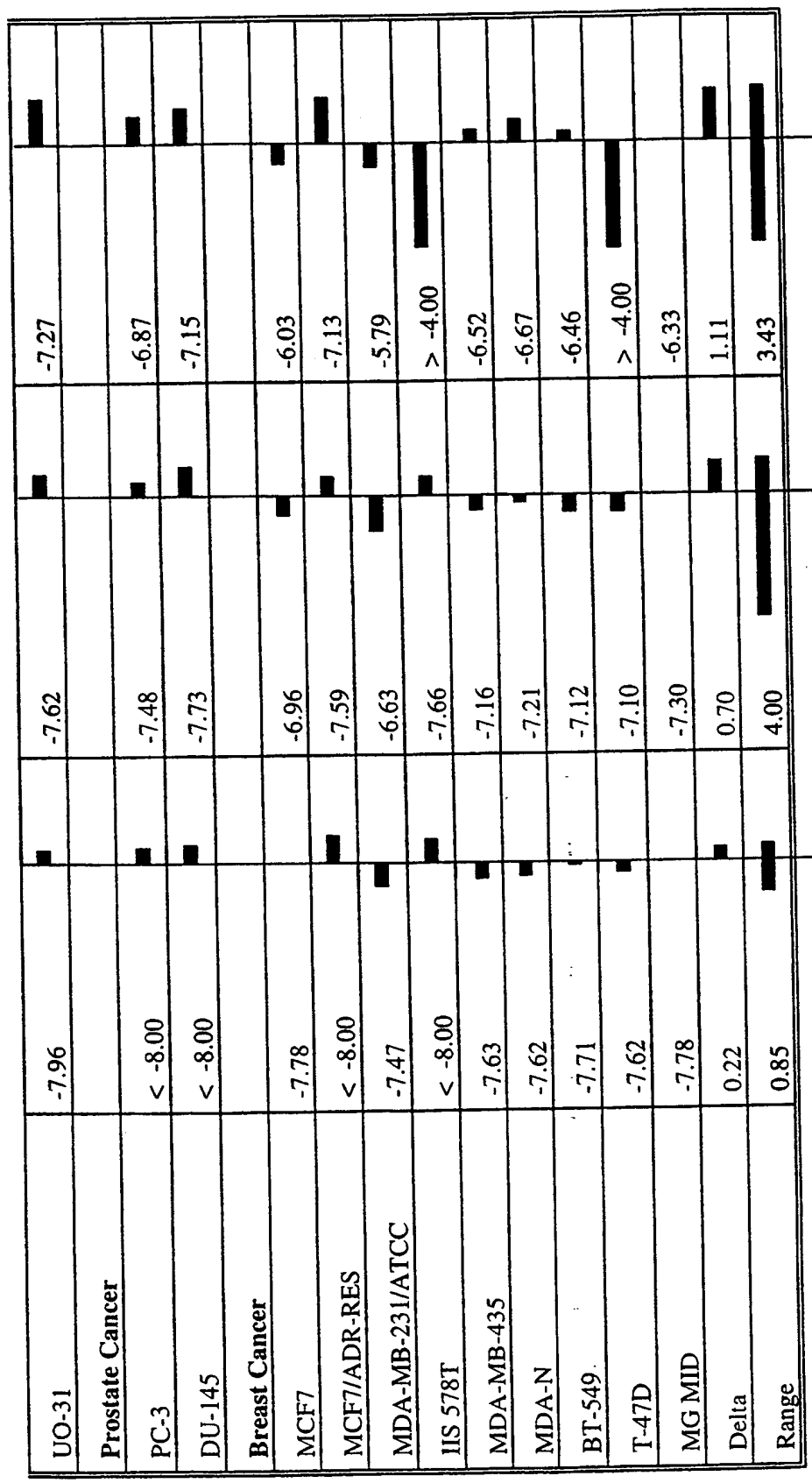

UO-31
-7.96
-7.62
-7.27
Prostate Cancer
PC-3
< -8.00
-7.48
-6.87
DU-145
< -8.00
-7.73
-7.15
Breast Cancer
MCF7
-7.78
-6.96
-6.03
MCF7/ADR-RES
< -8.00
-7.59
-7.13
MDA-MB-231/ATCC
-7.47
-6.63
-5.79
IIS 578T
< -8.00
-7.66
> -4.00
MDA-MB-435
-7.63
-7.16
-6.52
MDA-N
-7.62
-7.21
-6.67
BT-549
-7.71
-7.12
-6.46
T-47D
-7.62
-7.10
> -4.00
MG MID
-7.78
-7.30
-6.33
Delta
0.22
0.70
1.11
Range
0.85
4.00
3.43
+3 +2 +1 0 -1 -2 -3
+3 +2 +1 0 -1 -2 -3
+3 +2 +1 0 -1 -2 -3

Fig. 3m

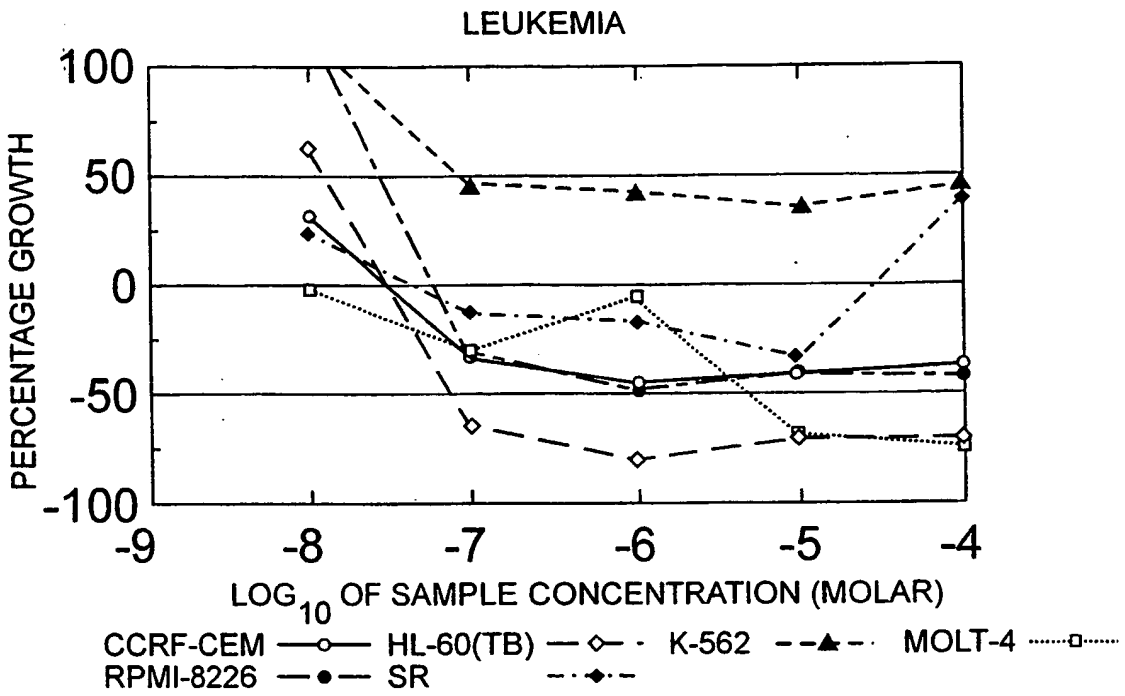

LEUKEMIA
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
CCRF-CEM
HL-60(TB)
K-562
MOLT-4
RPMI-8226
SR

Fig. 4a

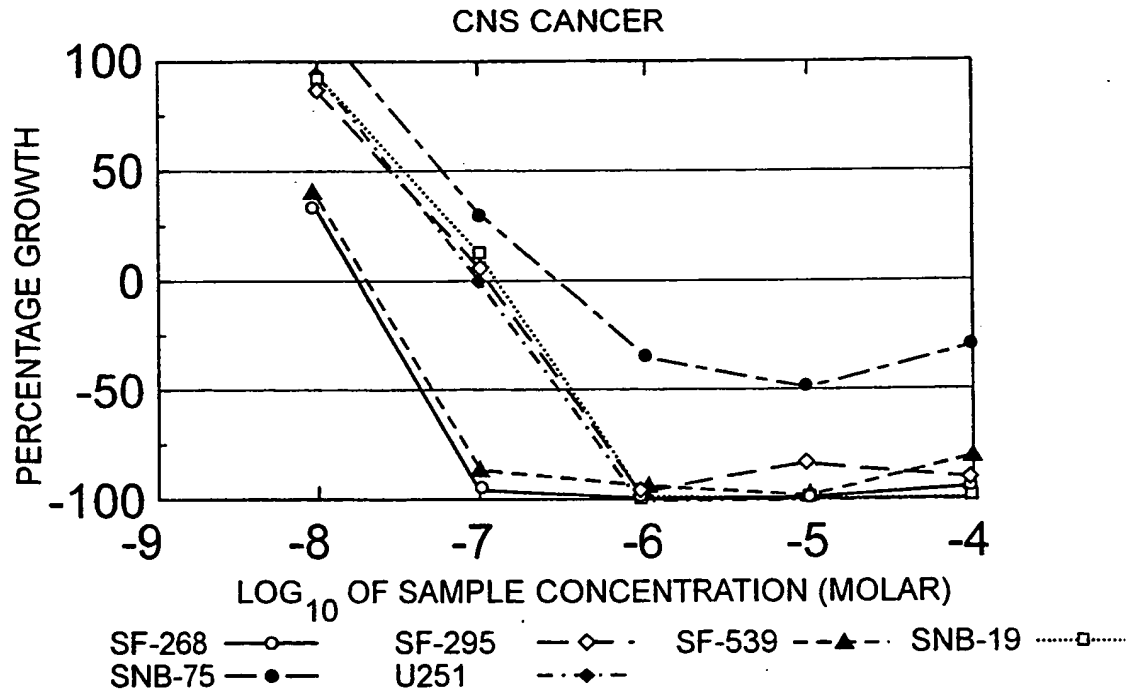

CNS CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
SF-268
SF-295
SF-539
SNB-19
SNB-75
U251

Fig. 4b

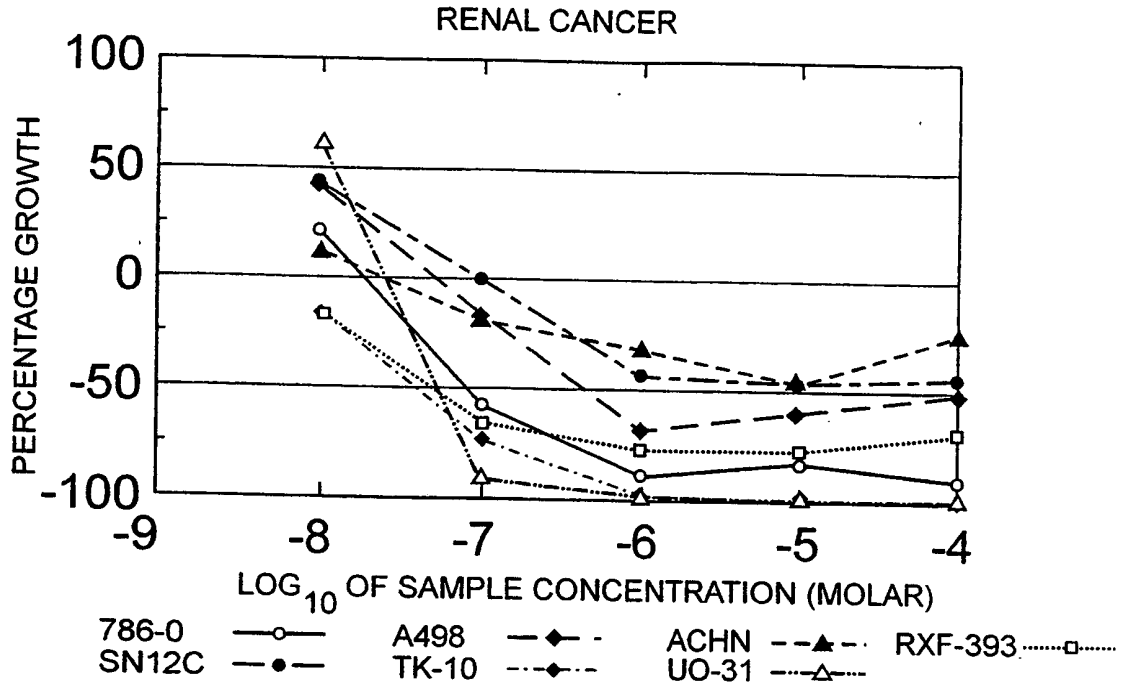

RENAL CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
786-0
SN12C
A498
TK-10
ACHN
UO-31
RXF-393

Fig. 4c

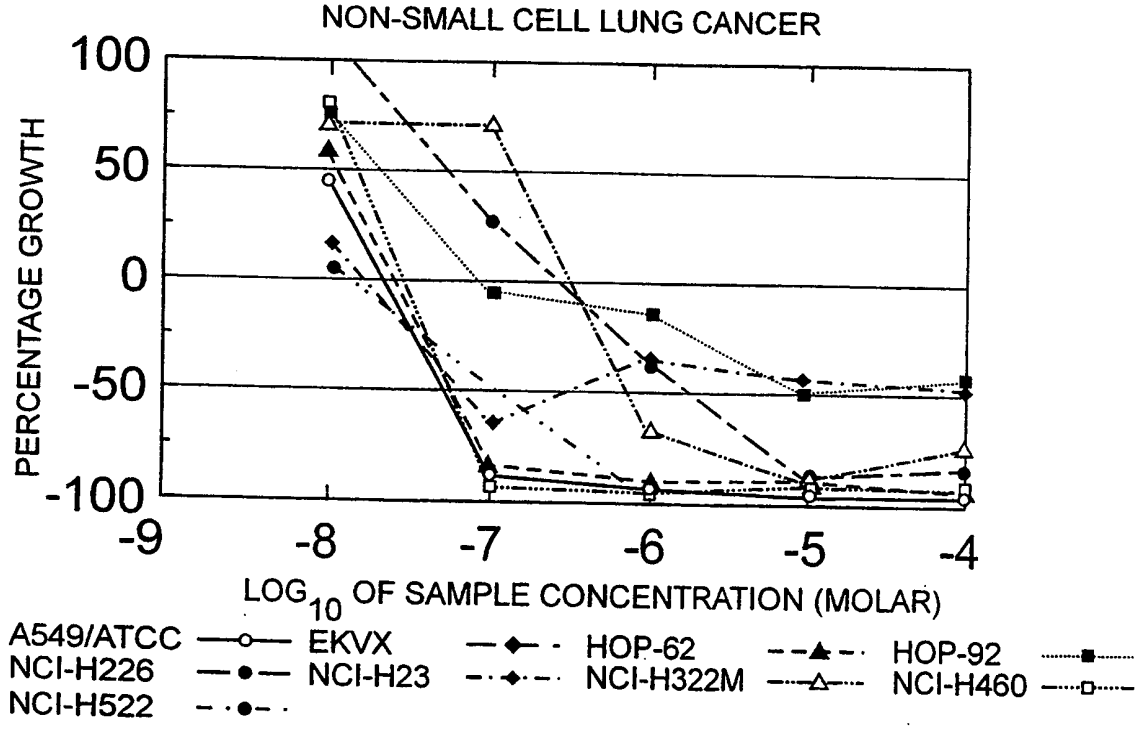

NON-SMALL CELL LUNG CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
A549/ATCC
EKVX
HOP-62
HOP-92
NCI-H226
NCI-H23
NCI-H322M
NCI-H460
NCI-H522

Fig. 4d

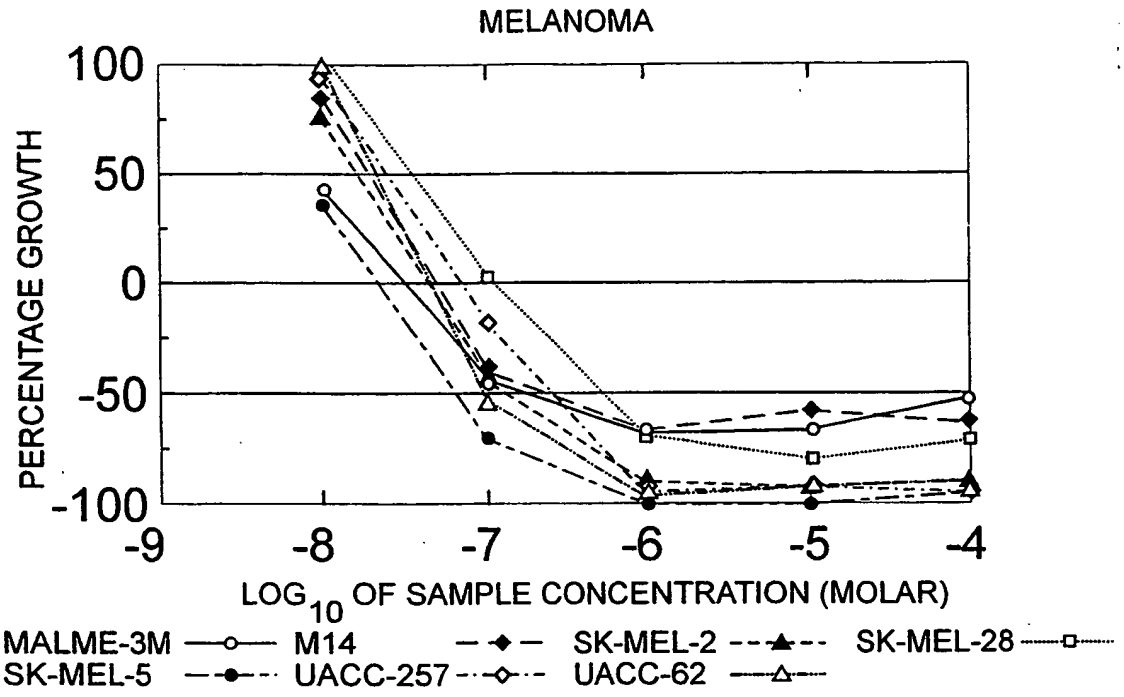

MELANOMA
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
MALME-3M
M14
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62

Fig. 4e

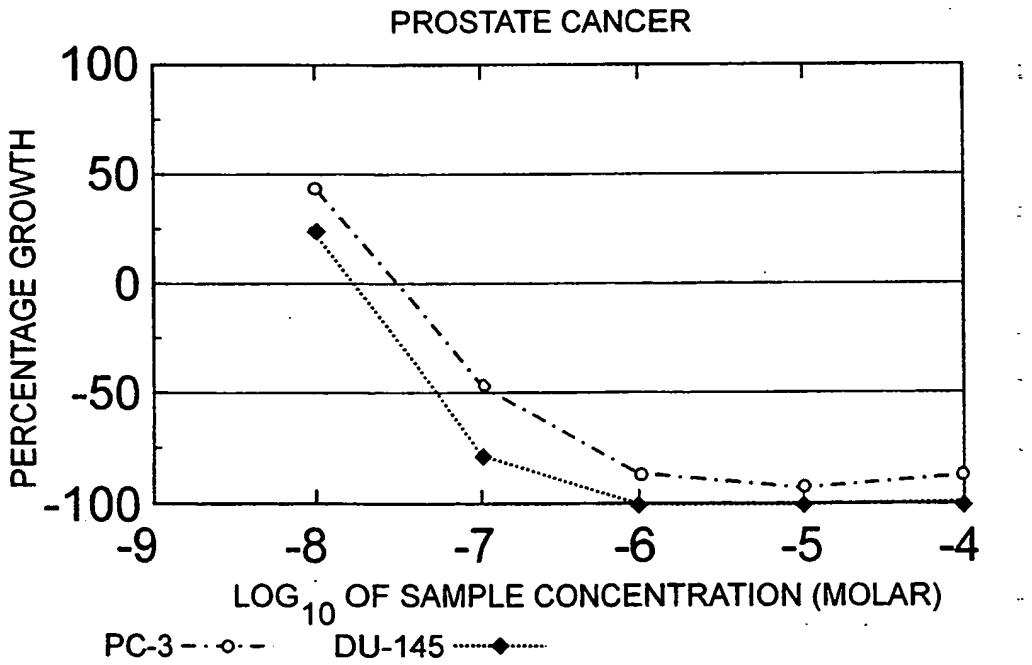

PROSTATE CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
PC-3
DU-145

Fig. 4f

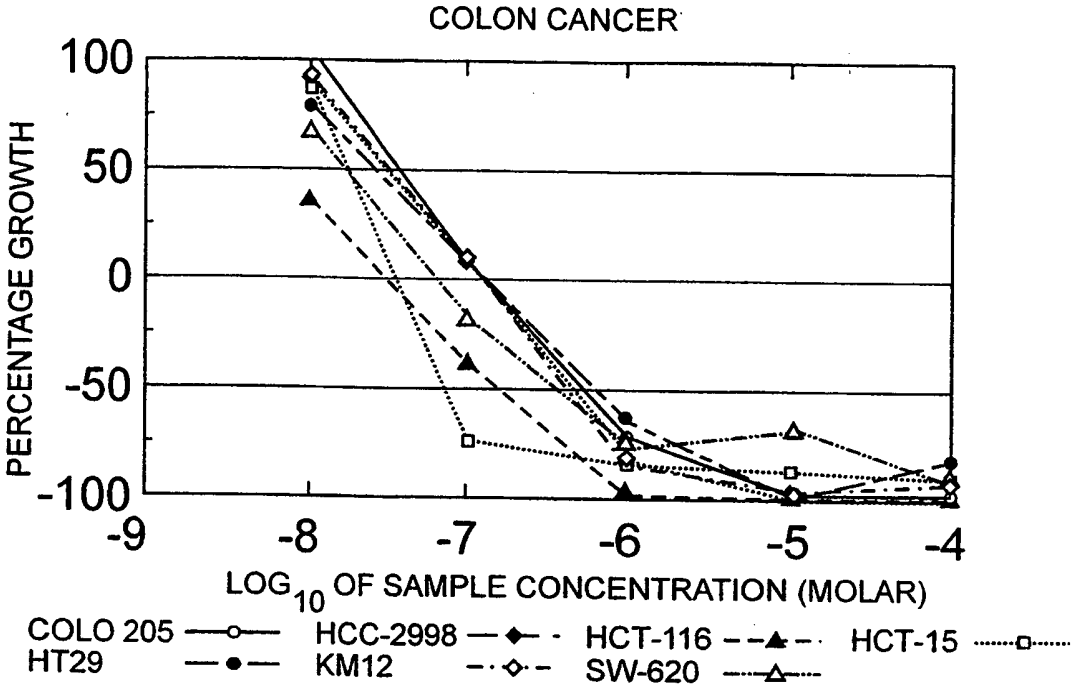

COLON CANCER
100
50
0
-50
-100
PERCENTAGE GROWTH
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
COLO 205
HCC-2998
HCT-116
HCT-15
HT29
KM12
SW-620

Fig. 4g

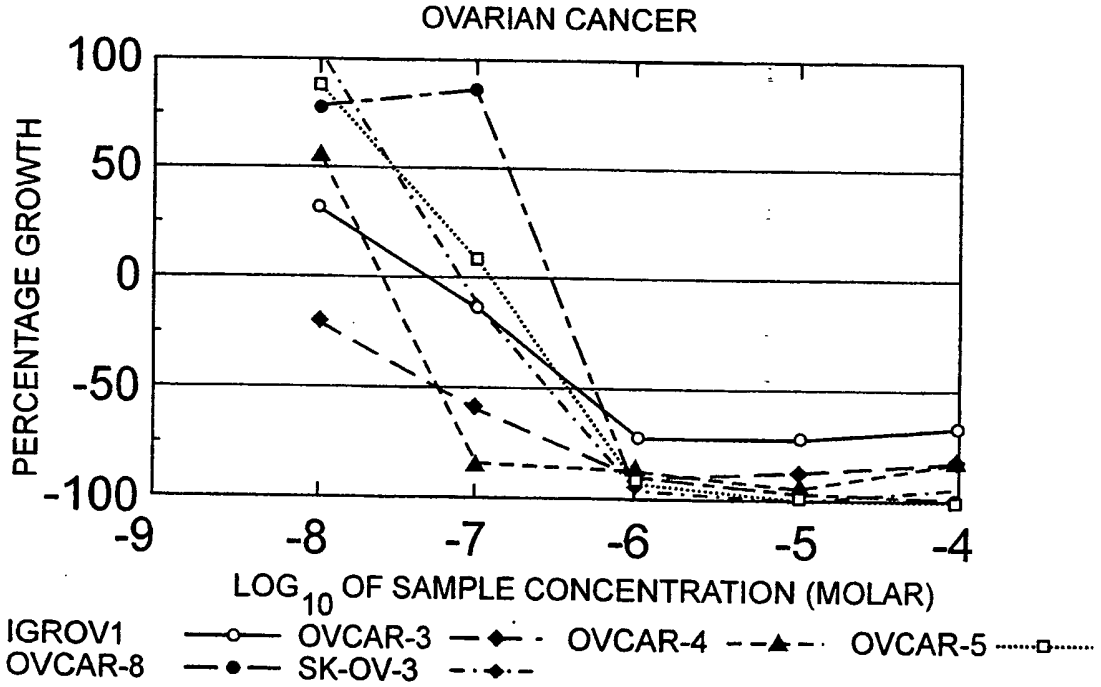

OVARIAN CANCER
100
50
0
-50
-100
PERCENTAGE GROWTH
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
IGROV1
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3

Fig. 4h

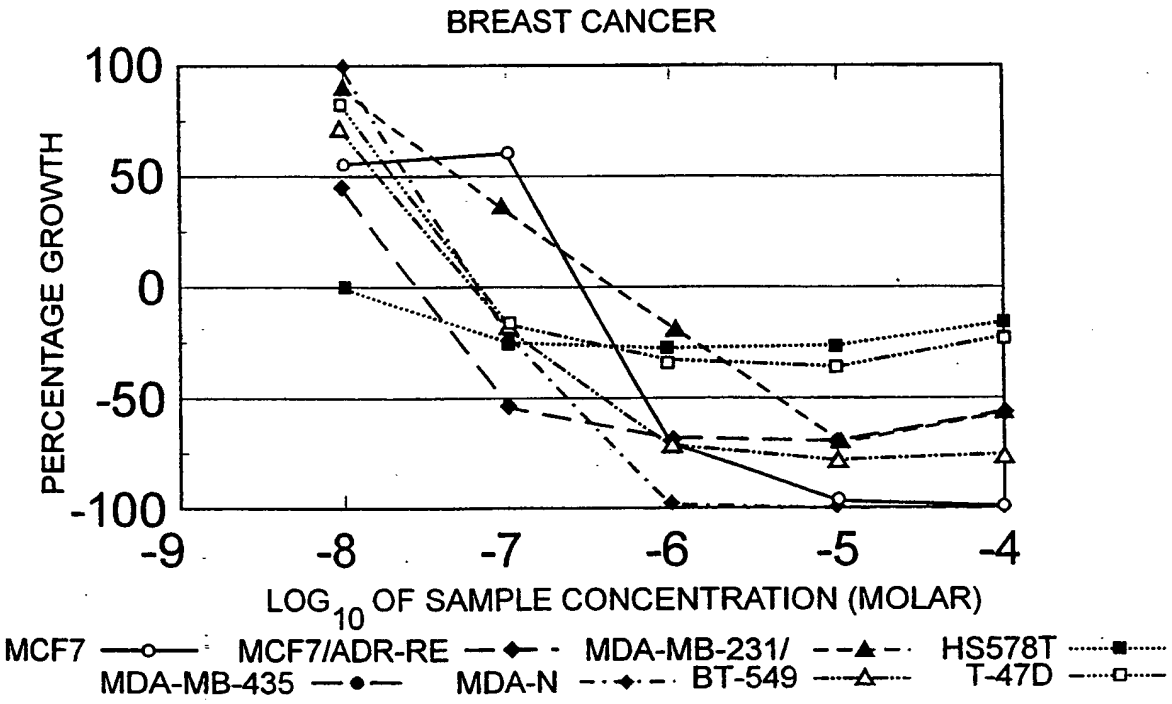

BREAST CANCER
PERCENTAGE GROWTH
100
50
0
-50
-100
-9
-8
-7
-6
-5
-4
LOG10 OF SAMPLE CONCENTRATION (MOLAR)
MCF7
MCF7/ADR-RE
MDA-MB-231/
HS578T
MDA-MB-435
MDA-N
BT-549
T-47D

Fig. 4i

National Cancer Institute Developmental Therapeutics Program	NSC: D-659472-Z/0-1/16	Units: Molar SSPL:	Exp. ID: 9302MD33
Mean Graphs - Calotoxin	Report Date: March 8, 1993	Test Date: February 23, 1993	
Panel/Cell Line	Log_{19} GI50 G150	Log_{19} TGI TGI	Log_{19} LC50 LC50
Leukemia			
CCRF-CEM	< -8.00	-7.51	> -4.00
HL-60(TB)	-7.93	-7.52	-7.10
K-562	-7.08	> -4.00	> -4.00
MOLT-4	< -8.00	< -8.00	-5.28
RPMI-8226	-7.57	-7.22	> -4.00
SR	< -8.00		> -4.00
Non-small Cell Lung Cancer			
A549/ATCC	< -8.00	-7.67	-7.29
EKVX	-7.95	-7.59	-7.22
HOP-62	-7.95	-7.60	-7.25
HOP-92	-7.69	-7.07	> -4.00
NCI-H226	-7.28	-6.57	> -4.00

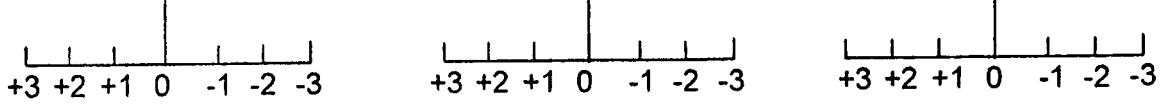


Fig. 4j

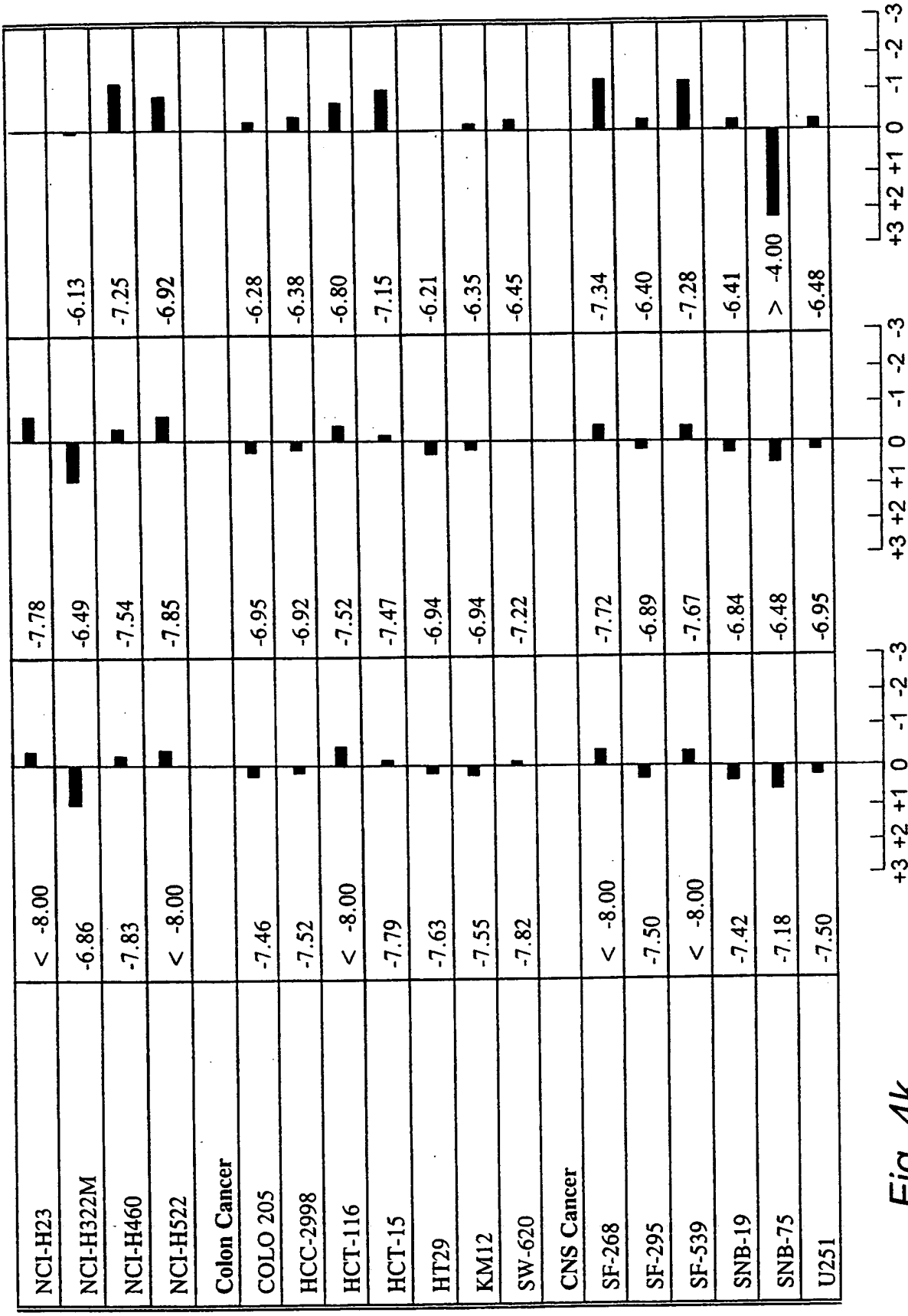

NCI-H23 < -8.00 -7.78
NCI-H322M -6.86 -6.49 -6.13
NCI-H460 -7.83 -7.54 -7.25
NCI-H522 < -8.00 -7.85 -6.92
Colon Cancer
COLO 205 -7.46 -6.95 -6.28
HCC-2998 -7.52 -6.92 -6.38
HCT-116 < -8.00 -7.52 -6.80
HCT-15 -7.79 -7.47 -7.15
HT29 -7.63 -6.94 -6.21
KM12 -7.55 -6.94 -6.35
SW-620 -7.82 -7.22 -6.45
CNS Cancer
SF-268 < -8.00 -7.72 -7.34
SF-295 -7.50 -6.89 -6.40
SF-539 < -8.00 -7.67 -7.28
SNB-19 -7.42 -6.84 -6.41
SNB-75 -7.18 -6.48 > -4.00
U251 -7.50 -6.95 -6.48
+3 +2 +1 0 -1 -2 -3

Fig. 4k

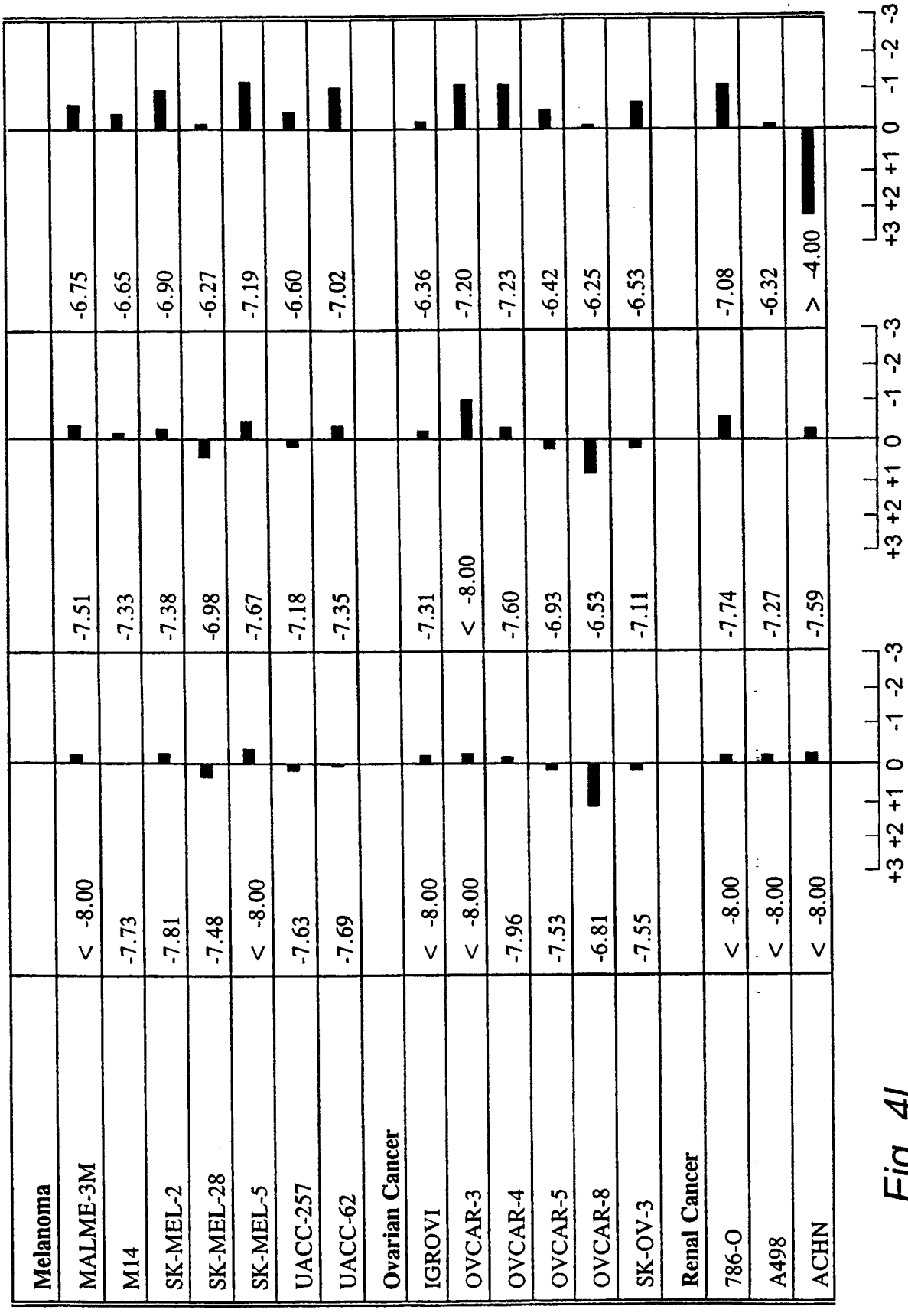
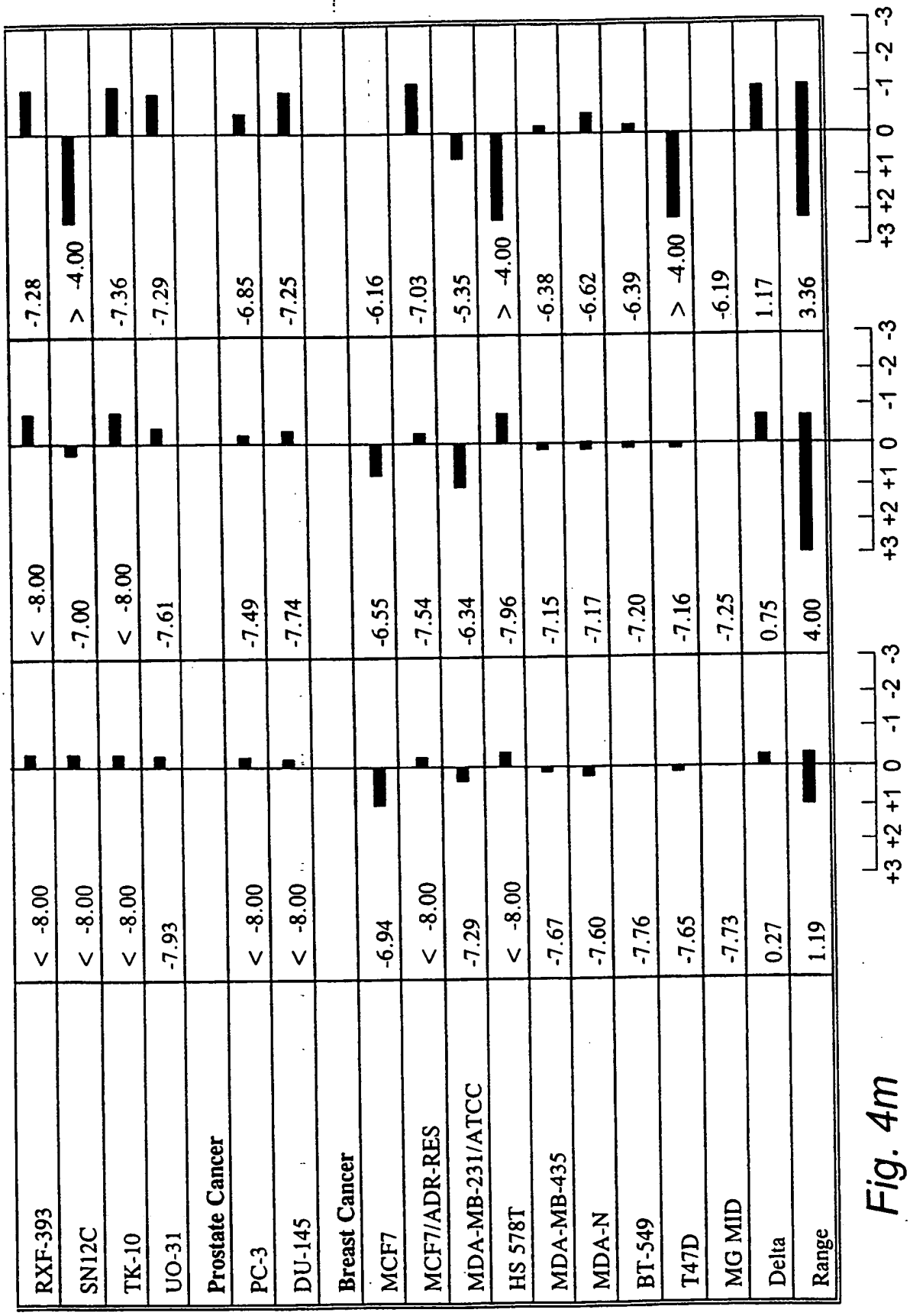

Melanoma
MALME-3M
M14
SK-MEL-2
SK-MEL-28
SK-MEL-5
UACC-257
UACC-62
Ovarian Cancer
IGROVI
OVCAR-3
OVCAR-4
OVCAR-5
OVCAR-8
SK-OV-3
Renal Cancer
786-O
A498
ACHN
< -8.00
-7.73
-7.81
-7.48
< -8.00
-7.63
-7.69
< -8.00
< -8.00
-7.96
-7.53
-6.81
-7.55
< -8.00
< -8.00
< -8.00
-7.51
-7.33
-7.38
-6.98
-7.67
-7.18
-7.35
-7.31
< -8.00
-7.60
-6.93
-6.53
-7.11
-7.74
-7.27
-7.59
-6.75
-6.65
-6.90
-6.27
-7.19
-6.60
-7.02
-6.36
-7.20
-7.23
-6.42
-6.25
-6.53
-7.08
-6.32
> -4.00
+3 +2 +1 0 -1 -2 -3

Fig. 4I



RXF-393	< -8.00	< -8.00		-7.28	
SN12C	< -8.00	-7.00		> -4.00	
TK-10	< -8.00	< -8.00		-7.36	
UO-31	-7.93	-7.61		-7.29	
Prostate Cancer					
PC-3	< -8.00	-7.49		-6.85	
DU-145	< -8.00	-7.74		-7.25	
Breast Cancer					
MCF7	-6.94	-6.55		-6.16	
MCF7/ADR-RES	< -8.00	-7.54		-7.03	
MDA-MB-231/ATCC	-7.29	-6.34		-5.35	
HS 578T	< -8.00	-7.96		> -4.00	
MDA-MB-435	-7.67	-7.15		-6.38	
MDA-N	-7.60	-7.17		-6.62	
BT-549	-7.76	-7.20		-6.39	
T47D	-7.65	-7.16		> -4.00	
MG MID	-7.73	-7.25		-6.19	
Delta	0.27	0.75		1.17	
Range	1.19	4.00		3.36	

+3 +2 +1 0 -1 -2 -3

Fig. 4m

INTERNATIONAL SEARCH REPORT

Int [illegible]ional Application No
PCT/[illegible]8/01522

A. CLASSIFICATION OF SUBJECT M[illegible]ER
IPC 6 A61K31/365

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.A. PARSONS: "Cat assay for the emetic action of digitalis and elated glycosides (digitoxin, digoxin, lanatoside C ouabain and calactin)" BR. J. PHARMACOL., vol. 42, no. 1, 1971, pages 143-152, XP002078318 see page 145 ---	1-8
P,X	F. KIUCHI ET AL.: "Cytotoxic priciples of a Bangladesh crude drug, akond mul (roots of Calotropis gigantea L.)" CHEM. PHARM. BULL., vol. 46, no. 3, 1998, pages 528-530, XP002078319 see the whole document ---	1-6
A	WO 92 09295 A (MRAK, M.,) 11 June 1992 ---	
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 September 1998	02/10/1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/[redacted]8/01522

C.(Continuation) DOCUMENTS CO[redacted]ED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	A.E.MUTLIB ET AL.: "In vivo and in vitro metabolism of gomphoside, a cardiotonic steroid with doubly-linked sugar." J. STEROID BIOCHEM., vol. 28, no. 1, 1987, pages 65-76, XP002078320	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]ional Application No
PCT/[illegible]/01522

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9209295 A	11-06-1992	CH 679012 A	13-12-1991
		AU 657283 B	09-03-1995
		AU 8902891 A	25-06-1992
		EP 0514508 A	25-11-1992

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